# THE AMERICAN JOURNAL OF PHYSIOLOGY

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## THE AMERICAN PHYSIOLOGICAL SOCIETY


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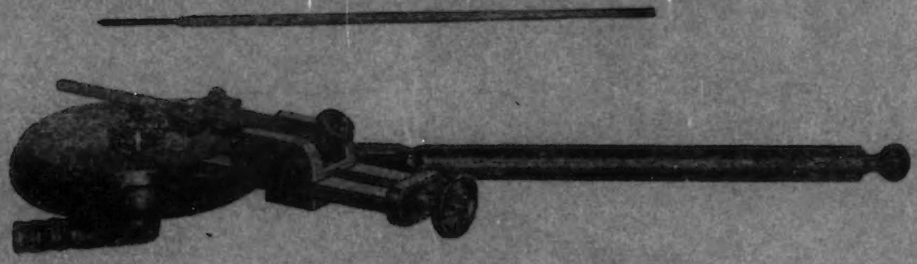

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THE AMERICAN
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# PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY

## Thirty-Fifth Annual Meeting

*Toronto, Canada, December 27, 28, 29, 1922*

*Experiments on the mechanism of action of insulin.* N. A. McCormick, J. J. R. Macleod, K. O'Brien and E. C. Noble.

When the percentage of blood sugar is measured at half-hour intervals following the injection of insulin in rabbits, the absolute fall is at first the same, within wide limits, whatever the dose of insulin. Neither the nutritive condition of the animals nor the method of administration (subcutaneous or intravenous) makes any significant difference in the steepness of the curve. The subsequent behavior of the curve depends partly on the dose of insulin and partly on the glycogen content of the liver. But even when these are the same in different animals, the curves may vary considerably, so that no accurate method of physiological assay can be based on measurement of the blood sugar at any chosen interval following injection. The rapid and uniform fall during the first half-hour is not due to increased intravascular glycolysis, since insulin has no effect on the rate of glycolysis in blood removed from the body. It is not due to polymerization of glucose in the blood, since hydrolysis of blood removed when the sugar is at its lowest does not cause any greater increase in reducing power than occurs under the same conditions in normal blood. The insulin must therefore enter the tissue cells and create therein a vacuum for glucose, as a result of which sugar is withdrawn from the blood more quickly than the liver and muscles can supply it from their stores of glycogen. Since insulin is known to cause marked glycogen formation in the liver of diabetic dogs, it is possible that the vacuum for glucose in the tissue cells is in part due to polymerization of glucose in them, but it is also partly due to increased combustion, since there is usually a marked increase in the R. Q. of normal, as well as diabetic dogs, and the oxygen consumption also increases (Dixon and Pember). The R. Q. may rise decidedly

above unity and remain there much longer than can be explained by a blowing off of $CO_2$ due to hyperpnea. This would seem to indicate a reduction of carbohydrate akin to that which occurs in hibernating animals during the fall months, while they are converting carbohydrate into fat. As the effect of insulin wears off, restoration of the sugar curve depends largely on the glycogen stores, but even in completely deglycogenated animals restoration also occurs, although more slowly, indicating that a process of gluconeogenesis is set up.

*Some chemical reactions of insulin.* C. H. BEST and J. J. R. MACLEOD.

Every sample of insulin prepared by the usual method from the pancreas of the ox or pig that has been examined by us, has given decidedly purple color by the Biuret test, an orange color by the Xanthoproteic test, and a faint purple (masked by browning) by the Hopkins Cole reaction. The intensity of the reactions has varied considerably with different preparations. On the other hand, insulin prepared from the pancreas of the skate by alcoholic extraction and heat gives none of the color reactions for protein. That the trace of protein in ox-insulin preparations is an impurity is evidenced by the fact that after precipitation with phosphotungstic acid and removal of the latter by ether and barium, the preparation regains its powers to cause hypoglycemia.

Insulin gives none of the usual color reactions for epinephrin.

Moderate heat does not appreciably affect the strength of insulin, at least in faintly acid reaction (pH between 5 and 6). Thus it may be heated on a boiling water bath for at least 10 minutes, and probably much longer, without perceptible alteration in strength, but it is destroyed by actual boiling for 3 minutes. Acidified hot water may therefore be used for the extraction of insulin from the pancreas.

Moderate degrees of alkalinity (to litmus) do not affect the strength of insulin during a period of 6 minutes at room temperature. We cannot as yet state the degree of alkalinity which insulin can withstand.

Insulin in acid reaction is absorbed by kaolin, charcoal, etc. Thus after shaking 4 cc. insulin with kaolin for 10 minutes, 0.8 cc. lowered the blood sugar from 0.104 to 0.060 in 1 hour, without symptoms, whereas 0.8 cc. of the original insulin lowered it from 0.098 to 0.037 in 1 hour, and caused convulsions.

It has so far been impossible to demonstrate any dialysis of insulin through collodion sacs, and usually there is also a marked reduction in the strength of insulin within the dialyser. These experiments have been conducted either by frequently changing the water outside the dialyser or by using a small volume of unchanged water. Dialysis has been allowed to proceed for 3 hours.

On several occasions it has been found that the potency of the insulin preparations is greatly reduced by passing through a Berkfeld filter. Thus: 1 cc. ox-insulin before Berkfelding lowered the blood sugar in 2 hours from 0.126 to 0.042, causing convulsions, and after it, from 0.130 to 0.072, with no symptoms; 3 cc. skate insulin before Berkfelding lowered the blood sugar in two hours from 0.120 to 0.054 causing convulsions, and after it from 0.122 to 0.090 with no symptoms.

By the method of formal titrations (Sorensen) it has been found that proteolytic enzymes are present in extracts of ox-pancreas after removal of the alcohol by the method described elsewhere, but none has been detected in the final preparations of insulin.

*Quantitative parallelism of effect of insulin in man, dog and rabbit.* H. G. BANTING, C. H. BEST, G. M. DOFFIN and J. A. GILCHRIST.

Over sixty preparations of "Insulin" made in the Connaught Antitoxin Laboratories and a number made by the Eli Lilly Company have been tested by intravenous or subcutaneous administration to normal rabbits. The fall in blood sugar in a period of 2 hours was determined in every case, and in a number of cases the blood sugar curve had fallen until it returned to its normal level. All of these preparations of insulin have been retested on diabetic patients at the Hospital of Department of Soldiers Civil Re-Establishment. Some of the preparations have also been tested on diabetic dogs in the Department of Pharmacology. As previously reported, there is a great variability in the reaction of normal rabbits to insulin. This variability is very noticeable in man and dogs. There is only slight co-relation between the relative lowerings of blood sugar in rabbits and in man with various lots of insulin. The area includes between the horizontal line drawn through the level of normal blood sugar, and the dip of the curve as outlined above gives a fairly accurate indication of the amount of carbohydrate a unit of insulin may be expected to enable a diabetic patient to utilize.

*Delayed manifestation of the physiological effects of insulin following the administration of certain pancreatic extracts.* J. B. COLLIP.

In the course of investigations on the preparation and purification of insulin, it was found that certain extracts which had been prepared and tested on normal rabbits produced no immediate hypoglycemia; in fact in many instances a decided hyperglycemia resulted. However, in a few instances it was noted that an animal which did not show a low blood sugar in the period immediately following the administration of the extract, did manifest a low blood sugar in from 1 to 4 days following the inoculation. Six examples of this delayed action are shown in the accompanying table. The first three observations occurred casually while the last three were definitely anticipated.

It would seem probable therefore that the active principle insulin does not exist in any quantity as such in the Islet cells. By certain methods of extraction one can produce a product which acts immediately after it is injected while by other methods of extraction a product can bese cured which, as far as its immediate effects on a normal animal are concerned, is relatively inert but which produces the same characteristic results after the lapse of many hours. These findings suggest to the writer that when an extract of pancreas is obtained which is highly potent, as indicated by the rabbit test, the potency of the extract is a result of the activation by certain of the reagents used of a mother substance resident in the normal gland. Delayed action,

on the other hand, would seem to be due to the extraction of the unaltered mother substance and the gradual activation of the same after the injection has been made.

| | | |
|---|---|---|
| June 20. | Normal rabbit | Blood Sugar = 0.094 |
| | 25 cc. of extract made from Mya. 1½ hrs. | Blood Sugar = 0.105 |
| | 25 cc. of extract made from Mya 2 hrs. later. | Blood Sugar = 0.130 |
| | Rabbit listless. 2 hrs. later | Blood Sugar = 0.180 |
| June 23. | 9 a.m. Animal in convulsions | Blood Sugar = 0.010 |
| Oct. 22. | Normal rabbit | Blood Sugar = 0.099 |
| | 12 cc. of an extract from Mya. *11 a.m.* 1½ hrs. later | Blood Sugar = 0.118 |
| | 6 hrs. later | Blood Sugar = 0.195 |
| Oct. 24. | Rabbit listless. *9 a.m.* Gave 51 grams sugar | Blood Sugar = 0.048 |
| Oct. 25. | Listless. *9 a.m.* | Blood Sugar = 0.01 |

*Methods of administration of pancreatic extracts to diabetic animals and men.* C. CLYDE SUTTER, C. B. F. GIBBS and JOHN R. MURLIN.

Reduction of the hyperglycemia and diminution of the glycosuria may be brought about by administration of pancreatic extracts directly into the duodenum of patients by the Einhorn tube. Administration of salol-covered charcoal tablets impregnated with concentrated extract in one instance gave slight protection. Concentrated extracts given by mouth have afforded no protection thus far unless given with sufficient alkali to neutralize the HCl of the gastric juice. Extracts given with sugar and alkali (N/20 NaOH) to depancreatized dogs raise the respiratory quotient as reported by Murlin and Kramer in 1916. Concentrated extracts of ox-pancreas have been prepared of sufficient potency to materially reduce hyperglycemia and glycosuria in severe cases of diabetes when administered subcutaneously in doses as small as ½ cc.

Numerous tables of data were presented before the society in support of these observations.

*Some effects of strychnine and physostigmine upon the blood picture.* C. W. EDMUNDS and PUTNAM C. LLOYD.

The administration of small doses of epinephrin produces a typical change in the relative numbers of the white blood cells. It was thought that possibly this reaction might be used as an index of increased activity of the adrenal glands and in addition perhaps furnish an explanation for some of the effects of two alkaloids; namely, strychnine and physostigmine, which have been shown to stimulate these glands. The administration to dogs of these two alkaloids is followed by an increase in the number of white blood cells which, as shown by the differential count, resembles exactly that produced by epinephrin. Removal of the adrenal glands from dogs and subsequent administration of the two alkaloids named shows an entire change in the blood picture. Instead of an increase in white cells, there is in every case a decrease so that the effects of these alkaloids in increasing the number of leucocytes are due to their stimulating the adrenal glands.

In addition to the changes in the white blood cells, physostigmine in still larger doses causes a marked increase in red cells, which increase resembles in all its reactions the increase produced by epinephrin with the exception that it is not prevented by the administration of ergotoxin. It is suggested that this reaction is produced by the physostigmine acting upon the bone marrow but not upon the same structure that is affected by epinephrin on account of the difference in relation to ergotoxin. With both epinephrin and physostigmine, it would seem not to be a true stimulation of the bone marrow but rather a pouring out of cells which are already formed in the bone marrow, but which have not yet been passed out into the general circulation.

*The significance of the dual function of hemoglobin in relation to the mechanism of the chemical regulation of respiration.* ROBERT GESELL.

Analysis of the correspondence between the composition of arterial blood and ventilation shows that hyperpnea may occur with alkaline as well as with acid blood, with alkaline blood of low as well as with high carbon dioxide tension, and with excess as well as with lack of arterial oxygen.

Hemorrhage and subsequent injection of gum-saline respectively increase and decrease ventilation. If this is due to changes in acidity of blood reaching the center, the center acts solely as rear guard to internal respiration, but unmodified blood which has already passed through the tissues when accelerated through the center may transform hyperpnea into apnea. Therefore, not only is the metabolism of the body as a whole a factor in the chemical control of respiration—the metabolism of the center itself is extremely important.

Decreased oxidation is probably associated with increased accumulation of acids in the tissues. In addition decreased oxygen consumption reduces the normal liberation of alkali by the hemoglobin as it passes through the tissues. There is consequently a broken coördination of the dual function of hemoglobin.

The increased response to the administration of carbon dioxide during oxygen want is due in large part to this broken coördination. Without denying the general toxic effects of high oxygen tension we also suggest that the increased response to carbon dioxide, and the hyperpnea and convulsions from excess of oxygen, are in part acidotic phenomena dependent on a similar broken coördination (failure of reduction of oxyhemoglobin due to excess of oxygen in the plasma).

The additive effects of reduced oxidation and the administration of carbon dioxide during hemorrhage, carbon monoxide poisoning and cyanide poisoning suggest similar stimulation.

In accordance with our view, anoxemia reduces oxidation in the respiratory center which leads to an accumulation of a relatively poorly diffusible acid in a relatively poorly buffered cytoplasm no longer fully protected by the potential alkalinity of the blood. By virtue of its own metabolism and the extreme sensitivity to minute changes in its own acidity the center is sensitive to minute changes in

its own oxidations and, therefore, may act as vanguard of internal respiration as well as rear guard.

Changes in oxidation constitute the indirect respiratory stimulus but in so far as oxidation determines the kind and amount of acid formed it is the normal regulator of respiration.

Our data and views we believe offer a basis for the explanation of hyperpnea associated with lack and excess of oxygen, with alkaline and acid blood, of the mechanism of stimulation of acid metabolites, of apnea, periodic and normal respiration, of the effects of forced breathing and of oxygen inhalation on suspended breathing, of acapnia and alkalosis of progressive anoxemia, of acapnia and alkalosis occurring during recovery from anoxemia, and of other respiratory phenomena.[1]

*Circulatory changes in experimental dehydration.* NORMAN M. KEITH. Rapid loss of water from the organism occurs in certain diseases. This fact is important in Asiatic cholera and acute anhydremia of infants. In these clinical conditions definite circulatory changes take place.

Woodyatt has demonstrated experimentally that marked water loss may follow the intravenous injection of various hypertonic solutions and that this water loss is often associated with a rise in body temperature. Some experiments carried out early in 1916 led me to believe that strong hypertonic solutions of saccharose when injected intravenously caused diuresis and circulatory changes without a rise in body temperature. This latter finding suggested an experimental method for studying in detail the circulatory changes that might occur in a dehydrated animal.

Normal dogs trained to lie quietly on the laboratory table were given intravenously a 50 per cent solution of saccharose, the actual amount injected being 8 grams saccharose for each kilogram of body weight each hour for 2 hours. The injection was given continuously by means of the Woodyatt automatic pump. No anesthesia was required. The urine was collected accurately by the use of a retention catheter. The body temperature was taken by rectum every half-hour. Initial control estimations were made of body temperature, hemoglobin, whole blood and serum viscosity, total blood and plasma volume and the arterial and venous oxygen content. Three to five hours after the injection was completed the water loss and decrease in body weight were determined. During this dehydration period a study of the circulatory changes was made. The animal was then permitted to drink measured quantities of water for the next 24 hours, the amount of water given being approximately that lost by diuresis the preceding day. At the end of this 24-hour period the body weight, the water retained and changes in the circulation were recorded.

During the injection period and the following 3 to 5 hours the hemoglobin in a unit volume of blood and the blood viscosity were determined at short regular intervals. This curve showed a definite paralell-

[1] Gesell, Capp and Foote: This Journal, 1922, lxiii, 1, 32.

ism between the changes in hemoglobin concentration and viscosity. There was an early fall in both but later a rapid marked rise which remained practically constant until the end of the dehydration period. Following the giving of a sufficient amount of water by mouth the viscosity and hemoglobin concentration fell to the normal levels.

The blood and circulatory changes observed when the dehydration was complete were characteristic and constant. The hemoglobin rose 19 to 28 per cent. The viscosity of whole blood and serum showed a definite constant rise. The percentage decrease in plasma volume varied from 24 to 44 per cent and the total blood volume 2 to 38 per cent. The percentage saturation of oxygen in the arterial blood remained at the normal constant level while the venous oxygen saturation was definitely decreased. The arterial blood pressure remained at the normal level. Following an adequate supply of water to the dehydrated organism the blood and circulation were rapidly restored to a condition approximately normal. The hemoglobin and viscosity decrease varied inversely to the increase in total plasma and blood volume. The venous oxygen saturation also rose.

The intravenous administration of saccharose in the amount and manner employed in these experiments offers a simple method for studying the effects of rapid dehydration in the living animal. An average loss of 11 per cent of the body weight can be practically all accounted for in the volume of urine excreted. Subsequent administration of water results in a rapid increase in body weight. Study of the circulation during the dehydration period reveals an increased viscosity and hemoglobin concentration, a distinctly diminished circulating volume of plasma but a normal arterial blood pressure. The circulation is rapidly restored to normal with the giving of an adequate amount of water. In these experiments there was no appreciable change in body temperature. This result would indicate that the temperature changes attending dehydration apparently are not entirely due to water loss per se. The rate of dehydration is equally if not more important.

*Experimental lead pallor.* TORALD SOLLMANN.

The comb and wattles of roosters give a striking demonstration of pallor following the oral administration of metallic lead. The phenomenon is illustrated by Lumiere color-photographs. The pallor is accompanied by severe fall of the red blood count, which sets in very early, and proceeds rapidly to a minimum of 60 to 75 per cent of the normal count. This is reached in 7 to 10 days, and then does not fall further, although the animal continues to lose weight until death, which occurs in 9 to 45 days, from inanition. The rapid fall in the corpuscles indicates that the anemia is due to destruction rather than to deficient production.

*A method of studying peristalsis in situ.* TORALD SOLLMANN.

The walls of the opened abdomen are suspended from a ring so that the abdominal cavity forms a bath for warmed Ringer's solution, in which

the intestines of the living animal perform very beautifully. Registration by means of levers may thus be supplemented by direct inspection, which is indispensable for the correct interpretation of the complex peristaltic movements. Drugs may be injected either into the intestinal lumen or into the circulation. The results appear to differ in some important particulars from those based on the pendulum movements of excised intestines.

*Filtration through frog-skin.* TORALD SOLLMANN.

The sacs formed by stripping the skin from the hind-legs of frogs are used to study the phenomena of filtration through a relatively simple living tissue, and for comparing these, with the same tissue in the dying and dead condition. Some of the results will be described.

*On the existence of a hitherto unknown dietary factor essential for reproduction.* HERBERT M. EVANS and K. SCOTT BISHOP.

When rats are reared on a so-called "basic" ration of casein (18) cornstarch (54) lard (15) milk fat (9) and salts (4) to which daily doses of 0.4–0.6 gram dried whole yeast are added, they grow normally but cannot reproduce. They exhibit normal oestrus and ovulation and conceive. The placentae are abnormal and the products of conception are invariably resorbed. Natural foodstuffs contain a substance, *X*, which prevents such a sterility or cures it, once established. Green leaves, the cereals, or fresh meat may thus rather suddenly restore fertility. In some cases the favorable result was secured when the new foodstuff was added to the basic ration, after ovulation and fertilization had occurred so that presumptive evidence is secured of normal germ cells but defective uterine function as the specific cause of the disease. There is a definite though low quota of the needed substance, *X*, in a high proportion of milk fat for when this constitutes 24 per cent of the diet, fertility may be secured. The new factor is distinct from *A* since it was very low in a particular specimen of cod liver oil of proven high *A* content and furthermore the dietary placental disease does not occur when the *A* quota is lower than in our basic ration, providing *X* be present. The water soluble vitamine B can not be concerned since when this factor is further increased by high yeast dosage or additional daily feeding of 10 cc. of fresh milk, the disease is not affected. Vitamine C seems definitely eliminated by the ineffectual outcome of daily fresh orange juice dosage and by the effectual results with cereals notoriously low in or devoid of C. It is remarkable that both milk in its fresh fluid state and the total milk solids are ineffectual in a curative or prophylactic sense even when constituting a third of the diet. Studies are being conducted on the characteristics of the factor indispensable for reproduction and on its further distribution in natural foods.

From this evidence alone it would be difficult to show that the early reproductive steps are normal and only the placentae diseased. Clearer indications that this is the truth are afforded by instances in which a

change in dietary régime effected after ovulation and fertilization had occurred, led to the birth of normal young.

Natural foodstuffs contain a substance, *X*, which prevents such a sterility or which cures the disorder brought on by the basic diet. Fresh meat, green leaves, and the cereals (wheat, oats) have thus been found to contain something which restores fertility. Milk fat has a low but appreciable quota of the needed substance for when it is made to constitute a fourth of the diet by weight, fertility may similarly be restored. Cod liver oil is low or lacking in the factor *X*. If diets be employed containing the *X* factor (cereals, meat, etc.) but lower in fat soluble vitamine A than is our basic ration (9 per cent), reproduction may be hindered by gonodal abnormality, but if conception occurs, there does not occur the characteristic resorption of the gestation peculiar to this disorder. It is hence clear that the substance *A* is not involved. The vitamine B was adequate in our basic ration as shown by normal growth and oestrous rhythm; but when B is further increased by large yeast dosage or 10 cc. daily of fresh milk, the disease is not affected. Vitamine B would thus also seem to be clearly eliminated from the problem. Daily doses of 8 cc. of fresh orange juice do not usually cure or prevent the disease, so that the antiscorbutic factor C could similarly be segregated. It is remarkable that both milk as fluid and the total milk solids fail in curative or prophylactic effect. The distribution and properties of the new substance, indispensable for reproduction, are being studied.

*The respiratory quotient and basal metabolism following removal of the liver and glucose injection.* FRANK C. MANN.

The respiratory quotient and basal metabolism were determined in a series of dogs before and after removal of the liver and the injection of glucose. The results showed that carbohydrate was being utilized after hepatectomy and the injection of glucose. This would suggest that the hypoglycemia following hepatectomy is due to the utilization of glucose and that the characteristic symptoms associated with hypoglycemia might be due to a lack of such utilization.

*Preliminary report on the daily amount, physical properties and rate of secretion of human pancreatic juice.* A. B. LUCKHARDT, FRED STANGL and FRED C. KOCH.

The subject for this study developed a pancreatic fistula follow an operation at Cook County Hospital on a gastric ulcer which h perforated into the lesser peritoneal sac. The man was in splend physical condition when first seen 1½ months after operation except for the small fistulous opening in the anterior abdominal wall. A suitable method was devised for the collection of all juice escaping from the fistula during a 24-hour interval. He was on the regular hospital diet. In addition, the patient ate food brought in by friends.

The following are the more important results obtained from this study:

1. Human pancreatic juice when first secreted is a colorless, limpid liquid much like distilled water. In reaction strongly alkaline to litmus. Its specific gravity (12 samples) averaged 1009; the average delta of the same samples was 0.625.

2. There was a continuous secretion (before breakfast) which amounted to 7.3 cc. to 8.5 cc. per hour (10 experiments).

3. Stimulated to prompt and marked activity by the ingestion of breakfast, the pancreas was highly active throughout the day, reaching its maximum secretion rate at 3 p.m. (breakfast at 7 a.m.; *dinner* at 12 m.; light lunch at 6 p.m.)

4. The average amount of pancreatic secretion collected in one day was 667 cc. (Extremes: 510 cc. and 862 cc.)

5. The lowest total output of secretion occurred on days when moderately large doses of alkalis were given, namely, 459 cc. (Extremes: 444 cc. and 474 cc.)

6. The maximum rate of secretion per hour was 84 cc. The average hourly rate of secretion (10 experiments) at its height (3 p.m.) was 54 cc. At the height of digestion the pancreas can, and sometimes does, secrete in one hour an amount of pancreatic juice equivalent to its own weight. At that, we have evidence that a great deal of pancreatic juice escaped into the duodenum.

7. After the ingestion of corresponding amounts of water, HCl, fat and carbohydrate, the maximum rate of secretion was obtained at the end of the first hour; in the case of protein, at the end of the third hour.

*The respiratory exchange under ether narcosis.* THEO. KRUSE.

Some years ago experiments were published from this laboratory (Guthrie, Journ. Amer. Med. Assoc., 1917, lxix, 1394) in which the respiratory exchange was studied upon animals in various stages of reduction and shock. At that time suitable means for the removal of ether from expired air was not available. Now that such a method has been developed, the respiratory exchange was studied upon anesthetized animals under variable experimental conditions.

Dogs were anesthetized with ether. Blood pressure, pulse, respiration rate, amplitude and volume were recorded and the composition of expired air analyzed for ether, carbon dioxide and oxygen. Experimental tests were made at selected intervals ranging in duration from 7 to 16 minutes. Depth of anesthesia was varied and in the reduced states (shock) the ether was removed.

The results were in accord qualitatively with previous observations on animals in reduced states, in which the ether factor had been inadequately controlled. After the induction of ether anesthesia the oxygen consumption falls but less intensely than the carbon dioxide elimination after which the latter falls more slowly or remains constant while the oxygen consumption continues to decrease.

Removal of ether leads to a prompt increase of respiratory exchange, the carbon dioxide elimination increasing more rapidly and to a greater

degree than the oxygen consumption. In such a stage the sum of the carbon dioxide and oxygen percentages may go as high as 21.60 per cent instead of a figure less than 20.90 per cent. The respiratory quotient at the same time may reach a figure as high as 1.45. If the animal has been sufficiently reduced so that the ether may remain off for a period ranging from 40 to 60 minutes, coincident with the the return of reflexes a stage is reached in which the carbon dioxide elimination is decreased while the oxygen consumption is on an increase. At this time the respiratory quotient falls to less than 1.0 and the sum of oxygen and carbon dioxide percentages is less than 20.90. Animals in this seemingly recovered stage require very little ether to reduce the respiratory exchange again to its previous low level.

*On the local reflex mechanism and the nature of the segmental contractions in the small intestine.* ALBERT KUNTZ and J. EARL THOMAS.

The present study includes experimental observations on the longitudinal and circular muscle in the small intestine (dog and cat) in intact animals under anesthesia in excised pieces of intestine immersed in Locke's solution, and in segments of the intestine perfused in situ.

Rhythmic contractions persist in the presence of nicotin hydrochloride in concentrations (0.1 to 0.2 per cent) sufficient to eliminate response to adrenalin and pilocarpin and to electrical stimulation of post-ganglionic sympathetic fibers. Consequently, they persist following paralysis of the most peripheral nervous element, the myoneural junction. Such contractions are not neurogenic. The results of these experiments on segments of the intact intestine corroborate the findings of Magnus ('05), Gunn and Underhill ('14), and Alvarez and Mahoney ('22), regarding the behavior of isolated strips of denervated intestinal muscle. They do not demonstrate the freedom of rhythmic segmentation of the intestine from nervous influence under normal conditions. Indeed, the tonus of the muscle and the amplitude and regularity of the contractions are definitely modified by this method of denervation, although the rate remains practically unchanged.

Inasmuch as the rhythmic contractions of the intestinal muscle persist following the elimination of nervous influence, an adequate explanation of all the motor activities of the small intestine requires neither axon reflexes nor any nervous mechanism other than the local reflex system in the enteric plexuses, connected with the central nervous system through the visceral afferent and efferent chains. The occurrence of reflex arcs in the enteric plexuses has recently been demonstrated histologically by one of the writers (Kuntz '22).

*The basal metabolism of girls from eleven to fourteen years of age.* MARY SWARTZ ROSE and GRACE MACLEOD.

Using the Benedict Portable Respiration Apparatus to determine oxygen consumption, basal metabolism determinations have been made on 34 subjects, 4 eleven-year-olds, 15 twelve-year-olds, 10 thirteen-year-olds and 5 fourteen-year-olds. Seven of the children have been followed

through 2 years, determinations being made every 6 months. In all cases at least two observations 6 months apart have been made, and for the sake of system each child every 6 months is counted as a new individual. That the children were healthy normal individuals has been determined from the physical examinations of the Horace Mann School from which they all came.

Weight, standing height and sitting height, respiration and pulse rates and vital capacity have been determined.

The ranges of the returns on the metabolism at different ages are as follows:

| AGE | CALORIES PER SQUARE METER PER HOUR |
|---|---|
| 11 | 35.9–42.5 |
| 12 | 31.3–51.6 |
| 13 | 35.9–49.1 |
| 14 | 31.8–41.4 |

These are discussed and compared with other figures for the same ages.

*The electrolytic conductance of tissues as affected by the surrounding medium.* S. C. BROOKS.

The concentration of electrolytes bathing various unicellular organisms was changed during the course of an experiment and it was observed that the relative conductance of the suspension of organisms and the suspending fluid remained the same. The same behavior was exhibited by tissue from the thallus of *Laminaria*. These experiments demonstrated that the concentration of conducting substances within the tissue was kept approximately proportional to that outside.

To determine whether the protoplasm takes part in this equilibrium, red blood cells were studied in the same way, being transferred from one solution to others of concentrations between one-half and five times the original one. The ratio of conductances was again approximately constant, and the red blood cells, which may be considered to be essentially living material, therefore compensate by changes of internal conductivity for similar changes in the environment.

*Cardiac size and output in man during rest and moderate exercise.* WALTER J. MEEK and J. A. E. EYSTER.

The cardiac size and output has been determined in 17 individuals during rest and moderate exercise by means of the x-ray. X-ray photographs of about $\frac{1}{10}$ second exposure were made during the R wave of the electrocardiogram, and at the end of the T wave. From the silhouettes the volumes were computed by Bardeen's formula.

In 10 individuals the diastolic size showed an increase during exercise, and in 7 a decrease. In 11 individuals the systolic size showed a decrease during exercise and in 6, an increase. The output per beat at rest averaged 79.4 cc. The output per beat in exercise averaged 94 cc. In 3 individuals there was an actual decreased output during exercise,

the minute output being increased by rate alone. The minute volume output averaged 5,914 cc. at rest, and during exercise rose to 10,750 cc. It will be noted that these figures are somewhat above the averages reported by the nitrous oxide method.

The authors think that their data clearly substantiate the belief that in exercise the output of the heart may be increased either by a larger output per beat or by an acceleration of the pulse or by any combination of these two factors.

In certain experiments there was an increased output per beat although the diastolic size had decreased. In such cases the increased stroke of the systole could hardly have been caused by an increased length of cardiac fiber in the preceding diastole. To meet such a situation one naturally postulates changes in tonus of the heart musculature.

*The physiological action of ionizing radiations.* ALFRED C. REDFIELD.

The $\alpha$, $\beta$ and $\gamma$ radiations from radium, x-rays and ultra-violet light have as a common physical property their ability to ionize matter. These otherwise diverse types of radiation influence the process of membrane formation in the egg of Nereis in an essentially identical manner. A theory is developed which has as its essential feature the assumption that the rate of physiological change is determined by an equilibrium between the rate of formation of ions, the rate of disappearance of ions in setting up the physiological process, and the rate of recombination of ions without causing physiological change. Mathematical treatment yields an equation which describes accurately the experimentally determined relation between intensity of radiation and the velocity of the resulting physiological reaction. It enables a number of predictions to be made concerning the relative effects of different kinds of radiation and concerning the effects of temperature on the velocity of the process. These predictions have been found by experiment to be fulfilled.

*On the cause of the increase in basal metabolism during the initial days of fasting.* MARGARET META KUNDE.

The reports of many of the earlier experiments on basal metabolism during fasting, lead one to believe that there is a decrease in the basal metabolic rate, throughout the entire fasting period, or especially striking during the first days of the fast. However, if one examines the data submitted from many of these experiments, one finds that instead of the decrease there is an actual increase in the basal metabolism during the first period of fasting.

This increase, though ignored in the interpretation of results, becomes obvious in the data published by such investigators as Senator, Munk, Benedict and others. Graphs plotted from their data show curves which point to an unquestionable rise in the basal metabolism during the initial days of fasting. This rise becomes strikingly apparent in the fasting studies o this report.

Since, in all cases, the subjects were fasting and presumably in complete muscular repose, the increased metabolism could not be attributed to the specific dynamic action of food or to voluntary muscular movements. Other experimental factors were likewise carefully controlled, so an increase due to environmental conditions may also be ruled out. There remains for consideration then, either chemical changes in the composition of the blood due to abnormal conditions of metabolism created by fasting, or changes in the tonicity of skeletal muscles brought about reflexly by afferent impulses from the empty gut.

If the afferent impulses from the empty alimentary tract are responsible for the increased metabolism, the increase should be prevented by the ingestion of non-irritating substances other than food by the fasting person. To determine this, the following series of experiments was performed. A man (F.H.), age 32, used and found reliable in previous fasting experiments voluntarily subjected himself to repeated short time fasts of 2 to 3 days. The normal basal metabolism was determined by the indirect methods for several days immediately preceding the fasts. Similar determinations were made at the same hour during the period of fasting. A marked rise in the basal metabolism occurred during the fasting days.

In another series the subject ate freely of "Cellu Flour" wafers so that at all times the alimentary tract was more or less filled with a non-nutritious, non-irritating mass. The "Cellu Flour" is obtained from purified and bleached wood pulp. It is tasteless and non-nutritive, containing no starch, sugar, fat or protein. In addition to the "Cellu Flour," the wafers contain small quantities of saccharine, india gum and mineral oil. The saccharine is added to make them more palatable, the India gum to give consistency, and the mineral oil in quantities favoring a normal daily bowel movement. The wafers were not baked but dried in a warming chamber heated to about 200°F.

With the subject fed on this non-nutritious mass, there is no rise in the basal metabolism during the initial days of fasting. There is also no definite lowering of the metabolism during these days.

To determine whether the bacteria of the digestive tract can produce fermentable substances from these wafers, the following fermentation tests were made. Six fermentation tubes, each containing 50 cc. of a 1 per cent solution of peptone were provided, to three of these tubes, 2 gram portions of the wafers were added. The other three tubes served as controls. Each tube was inoculated with four loopfuls of bacteria culture obtained as follows: A portion of feces about the size of a pea was thoroughly mixed with 10 cc. of a 1 per cent peptone solution. The solid material was allowed to settle to the bottom. The supernatant fluid containing the bacteria in suspension was used for inoculation. All tubes were then incubated at 37.5°C. for 3 days. At the end of that time a tiny bubble of gas about the size of a pin head appeared in one of the tubes containing wafer materiai, a similar amount was also present in one of the tubes containing only bacteria and peptone solution. All the other tubes were negative. The small amount of

gas formed is insignificant. The intestinal bacteria are unable to hydrolyze the gum and the cellulose in the wafers. The wafers are, therefore, free from nutrient material for man.

The increase in basal metabolism during the initial days of fasting in man can be prevented by the ingestion of non-irritating substances, other than food. The increased metabolism is probably due to afferent impulses from the empty and hypertonic gut, reflexly increasing muscle tone, rather than to chemical changes in the composition of the blood (acidosis) due to abnormal conditions of metabolism caused by fasting.

*Experimental production of peptic ulcer.* FRANK C. MANN and CARL S. WILLIAMSON.

Experiments were devised for diverting the secretions which neutralize the gastric juice as it leaves the stomach, to another portion of the intestine, removed from the point of emergence of the acid. Under such conditions typical subacute or chronic peptic ulcer developed in a high percentage of instances in the intestinal mucosa, just adjacent to the gastric mucosa. These ulcers appeared grossly and microscopically similar to the peptic ulcer noted in man. Thus we have developed a method for producing peptic ulcer which is quite comparable to that found in man.

*Vaccine fever in rabbits rendered poikilothermic by cervical cord transection.* ROBERT W. KEETON.

The cords of rabbits were transected beneath the sixth and seventh cervical vertebrae under local anesthesia (morphine-atropine injected subcutaneously, followed by infiltration with 0.2 per cent procain). In 3 to 4 days' time nutritional equilibrium was reëstablished and the animals were ready for the experimentation. The proof of poikilothermy was made by changing the environmental temperature through alterations in the amount of covering or the quantity of external heat. It is to be emphasized that during the experiment there were no appreciable changes in the humidity of the inspired air.

Some 4 to 5 hours after administration of food, with constant environmental temperature, rises in rectal temperatures of 1.5°C. to 3.6°C. were noted. The intravenous administration of single and repeated doses of stock typhoid para-typhoid vaccines to 4 such animals resulted in either no change or a drop in the temperature level, and a rapidly fatal issue. The subcutaneous and intra-muscular injections of single and repeated doses (1000 million to 2500 millions) of vaccines in 8 experiments on 7 animals gave definite persistent rises in temperature. If the animals were in poor nutritional state such as appears in the premoribund condition, no temperature rises occurred. Depending on the dosage of vaccine, the onset of fever began in 1 to 4 hours, and the height of the temperature varied between 1.2°C. and 2.9°C. with an average of 1.9°C. In 7 experiments the fever persisted over 12 hours.

*The production of acidosis by anoxemia.* A. E. KOEHLER, E. H. BRUNQUIST and A. S. LOEVENHART.

The view that decreased oxidation in the body leads to the accumulation of acid products has been held for many years and it has often been assumed that decreased oxidation leads to acidosis. Y. Henderson and Haggard questioned this conclusion and demonstrated a decrease in the H-ion concentration of the blood—i.e., alkalosis—as a result of short periods of decreased oxygen supply. He questioned whether decreased oxidation can produce acidosis. The alkalosis found by Henderson is due to the rapid excretion of $CO_2$ by the lungs.

It was the view of the writers that decreased oxidation does lead to acidosis and their views were put to test by the following method: Pigs were exposed to atmospheres low in oxygen with a low $CO_2$ content. The respiratory chamber, described by Kolls and Loevenhart, was used. The electrometric determination of the pH of the blood was made together with the total $CO_2$. The authors confirmed the findings of Henderson and Haggard and found a very definite increase in the pH of the blood from 7.4 to the region of 7.55 in the early stages of anoxemia. On continuing the anoxemia several hours, the pH returns to normal and continues to decrease. Marked increase in the respiratory rate begins as soon as the animals are exposed to low oxygen and continues throughout the experiment until near the end. At the beginning of the experiment the respiratory rate rises from the normal of approximately 40 to 200 per minute. There is but a short interval of time between the fall in the respiratory rate and death. The decrease in respiratory rate indicates exhaustion of the respiratory center.

At this time, we have frequently found the pH of the blood falls to 6.7 several minutes after the animal is removed from the chamber into the ordinary atmosphere. If the animal recovers, the return to normal is rapid—usually being complete in less than an hour, both in regard to blood pH and $CO_2$ content. The pH may then rise above normal (7.55). This alkalosis may last one hour but the normal is finally reached. At the height of acidosis the total $CO_2$ of the blood may fall to 10 per cent. The rise in $CO_2$ proceeds simultaneously with the rise in the blood pH.

The data prove the development of acidosis as a result of decreased oxygen supply. We believe that the production of fixed acids begins at once but does not keep pace with the loss of $CO_2$ from the blood so that alkalosis is first noted. Later the acid production more than compensates for the loss of $CO_2$ with the resulting very low pH values.

Various phases of this work are being continued with the coöperation of Messrs. H. M. F. Behneman, O. E. Benell and H. L. Schmitz.

*Effect of muscular activity on perfused adrenin.* R. G. HOSKINS and E. P. DURRANT.

Adrenalin chloride in calibrating doses (0.2–5 cc., 1:100,000 solution) was injected into the right common iliac arteries of dogs and cats in which the vagi and right sciatic nerves were cut. Carotid blood

pressure was recorded. The right leg was then severely fatigued by tetanizing the sciatic nerve. Standard doses of adrenalin were again injected. The systemic reactions to the adrenin were not decreased, hence none of the adrenin was taken up by the fatigued muscle. These results in the light of previous observations suggest that adrenin is a respiratory catalyst.

*Some conditions determining adrenal secretion.* F. A. HARTMAN.

Various stimuli applied to a cat with a completely denervated iris will cause dilatation of the pupil. Similar stimuli cause little or no dilatation of the completely denervated pupil after the adrenals are removed. This slight dilatation may be due to an increase in the temperature of the iris resulting from passive vascular dilatation. The completely denervated pupil, therefore, can be used to indicate increased epinephrin discharge and has so been used. The barking dog has produced very little if any increase in the output of epinephrin in the cats which we have studied.

Stimulation of the moist pinna by the inductorium causes a decided increase in epinephrin discharge, somewhat in proportion to the stimulus.

Complete asphyxia for 40 seconds produces a very great increase in epinephrin discharge.

Stimulation by cold causes a marked increase in epinephrin discharge.

The order of effectiveness in producing increased epinephrin discharge is, beginning with the weakest, dog barking, pinna stimulation, cold and asphyxia.

Surgical operations increase the output of epinephrin. Ether does not prevent the reflex secretion. Ether, by its direct effect, causes a small dilatation of the completely denervated pupil. Skin incisions usually cause a decided increase in epinephrin discharge. Cutting through muscle may cause a further increase. Manipulation of abdominal organs causes an even greater increase.

These observations indicate an emergency function of the adrenal.

*Action of digestive enzymes on pituitary extract.* MAURICE H. REES and RICHARD W. WHITEHEAD.

The authors determined the action of pituitrin, and pituitrin digested for varying periods with gastric and intestinal enzymes, on uterine contractions (isolated uterine strips), and with the latter in situ, and on blood pressure and respiration. The site and time of absorption of infundibular extract from the gastro-intestinal tract was also investigated.

With isolated uterine strips pituitrin mixed with enzymes caused some increase of tone, after this substance had been digested for varying periods. Results show that the uterine stimulating principle in pituitrin is only delayed in its effect by digestion for a half-hour or less, and is not destroyed by these enzymes. Enzymes probably do not entirely destroy the uterine stimulating principle. The effect of pituitrin is increased by digestion for an hour to 2 hours, above that resulting from the action of the same enzymes for a half-hour or less.

Enzymes given intravenously caused a fall in blood pressure. Pituitrin digested with enzymes resulted in a fall followed by the normal rise caused by pituitrin when given by vein. It was concluded from this that the pressor principle of pituitrin was not destroyed by digestive enzymes. Injection of pituitrin into various parts of the intestinal tract resulted in no rise of blood pressure, uniformly. Similar injections showed the uterine stimulating substance to be absorbed *entirely* from the *duodenum*. Absorption took place in from 50 seconds to 2½ minutes. The effect lasted over 15 minutes. Neither enzymes or pituitrin or mixtures of these had any effect on respiration in the dog.

*Neuro-muscular activity in cretinoid sheep.* SUTHERLAND SIMPSON and HOWARD S. LIDDELL.

The hypothyroid state in the human subject is characterized by subnormal temperature, slow heart beat, roughness and dryness of the skin and hair, stolidity, sluggishness, somnolence and lack of energy, and readily produced muscular fatigue. In the cretinoid sheep some of these signs are emphasized while others are less evident. Next to the characteristic stunting in growth, probably the most striking feature is the dull, stupid appearance of the animal and its stolid indifference to its surroundings.

In order to determine to what extent the spontaneous activity of the animal is affected by this condition a pedometer, calibrated to record the number of steps taken in the 24 hours (from which the distance covered could be approximately calculated) was attached to one of the forelegs.

In one series of eight pairs of twin lambs of the same sex (two pairs of males and six pairs of females), one of the members of each pair was thyroidectomized at the age of about 3 months. Four months after the operation while stunting was evident in several of the operated animals the muscular activity as compared with that of the control twin was not diminished.

In another series of four pairs of female twins 17 months after the removal of the thyroid glands (at the age of about 6 weeks), the cretinoid state was very evident, the weights of the operated animals being 30 per cent, 57 per cent, 63 per cent and 32 per cent less than those of the corresponding controls. Tested over a period of 19 days one of the cretins was only 70 per cent as active as the twin control, and showed greatly diminished muscular power, two of the others exhibited muscular weakness while the fourth showed neither pronounced muscular weakness nor greatly diminished activity.

*The cost of work in exophthalmic goiter.* H. S. PLUMMER and W. M. BOOTHBY.

Balanced total and basal metabolism studies from this laboratory showed that patients with exophthalmic goiter often require a daily food intake amounting to 5000 calories to maintain weight even when confined to bed. Since a large fraction of this food could not be accounted

for by the assumptions usually made for normal individuals in the calculation of food utilization, it appeared possible that an insufficient allowance was allotted for muscular activity. Accordingly a treadmill was constructed and the net cost of work determined by the gasometer method with the following results. Three normal individuals required an average of 1.18 gram calories as the net cost over the basal per horizontal kilogrammeter at the approximate rate of 800 meters per hour. Four patients in a debilitated condition had a similar average net cost of 1.18 gram calories per horizontal kilogrammeter. In contrast 6 patients with exophthalmic goiter under identical conditions of work required an average of 2.28 gram calories per horizontal kilogrammeter. One of these patients before operation with a basal metabolic rate of +51 per cent, required 2.16, 2.04 and 2.07 gram calories; 6 days after operation the basal metabolic rate had decreased to +25 per cent and the net cost of work had decreased to 1.73 and 1.75 gram calories per horizontal kilogrammeter.

The conclusion then is warranted that patients with moderately severe exophthalmic goiter are very inefficient in converting potential energy into any kind of motion as they require approximately twice as many net gram calories per horizontal kilogrammeter as normal individuals. This type of disease, therefore, is associated not only with a characteristic increase in the number of useless extraneous movements but further each movement requires approximately twice the normal amount of energy for its accomplishment. Muscular tissue with a resting metabolism increased above normal by thyroxin cannot utilize any portion of this extra heat for the production of motion.

*The calorigenic action of adrenalin chloride.* W. M. Boothby and I. Sandiford.

Fifty-eight metabolism experiments consisting of 3 to 7 periods each have been carried out by the gasometer method to determine the effect in trained, normal dogs of a regulated and constant intravenous injection of adrenalin chloride in normal saline solution. Many of these were control experiments to establish the constancy of the metabolism from period to period and from day to day. Six of the experiments were carried out as controls using a similar quantity of normal saline without the addition of adrenalin; all of these were negative. Various doses of adrenalin were used varying from 0.0023 mgm. to 0.0006 mgm. of adrenalin chloride per kilo per minute for periods of 6 to 12 minutes and in all instances an unquestionable increase in metabolism occurred.

These experiments seem to establish the theory first brought out by us at this meeting in 1919 that adrenalin chloride produces a direct chemical stimulation of cellular combustion and thus causes the cells to metabolize more rapidly under its influence. The evidence now available justifies the generalization that a function of the adrenal glands is to furnish a secretion of a catalytic nature which under normal biologic conditions accelerates the velocity of transformation of potential into kinetic energy.

*The effect of thyro-parathyroidectomy and parathyroidectomy at 100 days of age on the growth of male albino rats.* FREDERICK S. HAMMETT.

Growth in body length, body weight and tail length is retarded by the loss of the thyroid apparatus as a whole or by the parathyroids alone, but to a lesser degree by the latter.

Growth in body weight is retarded more than growth in body length or tail length. This is interpreted as signifying that growth by increase in cell number is more resistant to disturbing influences and hence more stable, than is growth by increase in cell volume, or growth by accretion.

The growth capacity of the parathyroidectomized rats showed alternate periods of marked decrease and increase, at times reaching the value to be expected on the basis of the observed body weight. Hence the growth capacity is not destroyed by parathyroidectomy, but is at times prevented from expression. The irregularity indicates the repeated accumulation and subsequent destruction or elimination of toxic products and a consequent checking and release of the ability to grow.

The growth capacity of the thyroidless rats does not exhibit such changes. It tends to gradually increase in value from the original low figure obtaining directly after the loss of the thyroid, until it reaches a state of equilibrium consistent with the lowered metabolism. Growth then proceeds as usual but at a lower level than that normal for the actual body weight.

The basis of the growth retardation after parathyroidectomy is therefore quite different from that occasioned by the loss of the thyroid. The former is a toxemia, the latter an actual diminution in the ability to grow.

*The pathogenesis of parathyroid tetany.* LESTER R. DRAGSTEDT.

In previous communications[1] it was reported that completely thyro-parathyroidectomized dogs could be kept alive, at least for a year, by diets containing large amounts of lactose. Such diets bring about a complete suppression of bacterial proteolysis in the intestine and the resultant production of intestinal poisons. It was concluded that parathyroid tetany or depression was due to an intoxication and that the responsible toxic substances come chiefly from the gastro-intestinal tract. They arise through the activity of the proteolytic group of intestinal bacteria, and are probably for the most part protein split products of the nature of amines. The function of the parathyroid glands is to prevent intoxication by these poisons. The parathyroid glands do not furnish a hormone necessary for life and dogs may be kept alive indefinitely after their removal if treatment directed to the prevention of this toxemia of intestinal origin is carried out. After about 6 weeks of dietary control, the parathyroidectomized dogs may be placed on usual stock diets without developing tetany or depression.

[1] Lester R. Dragstedt, This Journal, 1922, lix, 483; Journ. Amer. Med. Assoc., 1922, lxxix, 1593.

The mechanism of this readjustment to the loss of the parathyroids is not entirely clear but it seems most likely that some other organ, probably the liver, has taken over the function of the missing tissue. In two such recovered animals the production of a liver injury by the administration of phosphorus resulted in attacks of tetany exactly like that following parathyroidectomy. The readjustment in these recovered animals is not complete and they are in a state which in many respects resembles conditions of latent tetany and other spasmodic affections in man. A diet containing large amounts of meat, particularly if partly spoiled, may occasion a violent epileptiform convulsion in some animals or in others coarse jerking movements which remind one of paralysis agitans. The occurrence of constipation may have the same effect. Animals that have been in apparently excellent condition for many months with no tetany symptoms, may develop all of these coincident with the period of rut or heat, which is analogous to menstruation in primates. An exceedingly hot day, excessive exercise, excitement, or the occurrence of an infection (scabies) has precipitated an attack of tetany in these recovered animals. They are much less resistant to various convulsive poisons than are normal dogs. Doses of guanidine, methyl guanidine, trimethylamine, histamine, choline, neurine and physostigmine, less than one-fourth of that necessary to affect normal animals, will throw these into violent convulsions. Pregnant animals cannot be prevented from dying in tetany or depression, following parathyroidectomy, by dietary control. Apparently in this condition poisons are also absorbed from the uterus, arising from some of the products of conception.

*Additional observations on completely thyroparathyroidectomized dogs.* A. B. LUCKHARDT and J. BLUMENSTOCK.

The authors presented a résumé of the work which has appeared in several preliminary reports published during the past year[1] on the preservation of life of completely parathyroidectomized dogs (young, old, or pregnant) by timely and repeated intravenous injection of Ringer's solution in proper amounts as well as by the oral administration in comparatively large amounts of calcium lactate.

In one of these reports record was made of the fact (abundantly confirmed since then) that during the oestrus cycle all symptoms of parathyroid tetany recur. The severity of the symptoms at the first oestrus cycle following operation may even be more severe in a given animal than the symptoms seen immediately following thyroparathyroidectomy. It appears, however, that the symptoms of tetany become less severe at succeeding "heat" periods or may be entirely absent except possibly for a general increase in nervous excitability. In one animal, complete ovariotomy performed at the beginning of an oestrus cycle did not lessen to any noticeable degree the symptoms of parathyroid tetany.

As a distinctly new feature we wish to record the fact that the animals

[1] Proc. Soc. Exper. Biol. and Med., 1921, 129; Science, 1922, lvi, 48; Science, 1922, lvi, 257; Journ. Amer. Med. Assoc., Jan., 1923 (in press).

which have survived complete parathyroidectomy 8 months or more show, depending on the length of survival and the intensity of the post-operative tetany, more or less marked *bilateral cataracts*.

So far, completely and but recently thyroparathyroidectomized bitches appear not to be very fertile. All attempts, during the past year, to impregnate them have so far failed, in spite of the fact that various potent males were allowed to copulate 10 or more times with a given bitch. It is our *opinion* that this sterility is possibly an expression of thyroid deficiency or the result of a mild chronic intoxication interfering with normal gestation.

*Increase in size at molting.* J. P. BAUMBERGER and J. M. D. OLMSTED.

Crabs cast their hard exoskeleton at intervals, and while still soft absorb water sufficient to greatly increase their volume. The crab, when about to molt, can be easily recognized by the peculiarly friable condition of the carapace, always present at this time. A study of the freezing point depression of the body fluids of crabs at various stages in the molt cycle showed that the osmotic pressure is highest in the crab about to molt. Studies of water content showed that water absorbed after ecdysis is sufficient to account for the drop in osmotic pressure that takes place at this time. The high osmotic pressure preparatory to molting is not a gradual but rather a sudden development. The osmotically active compound is diffusible through an artificial membrane, and it is probably masked in the organism until the proper moment. Some polymerized compound could behave in this manner. Experiments are now under way to determine what chemical changes are responsible for the changes in oxmotic pressure. The table below gives the osmotic pressures for the different stages in terms of molar strengths of an ideal compound.

| CONDITION OF CRAB | OSMOTIC PRESSURE EXPRESSED IN MOLAR CONCENTRATIONS |
|---|---|
| Hard shell | 0.71 |
| About to molt | 1.40 |
| Newly molted | 1.18 |
| Paper shell | 0.94 |

*Retinal mean local sign: a new view of the relation of the retinal mosaic to visual perception.* F. W. WEYMOUTH, EMELIE E. ANDERSEN and HAROLD L. AVERILL.

Experiments in binocular perception of distance and monocular perception of the displacement of segments of a line have given retinal thresholds of from one-fourth to one twenty-fifth of a cone-diameter. Other workers have obtained thresholds of a similar order of magnitude, but none of the proposed explanations of how such small image-differences can be recognized are satisfactory.

Our view is that the visual stimuli give rise to a complex percept of position which we propose to call *retinal mean local sign*. At least three factors contribute to this: *1*, the mutual effect of adjacent retinal elements in either retina; *2*, the averaging of successive stimulus-patterns in each retinal mosaic caused by constant slight eye-movement; and *3*, the combining of the two simultaneous stimulus-patterns presented to the two eyes. In this view both the "straightness" and the position of a straight line are concepts of a high degree of complexity involving, in consequence, much more of the neural mechanism than the retina.

The mutual effect of adjacent cones would tend to straighten a linear image in an immobile eye. The eye is, however, in constant movement, and in consequence the image is swept across the retina giving rise to a succession of stimulus-patterns in which the irregularities incident to cone position take a variety of forms. The image of a straight line would, therefore, be presented to the brain as a series of irregular lines, the sinuosities of which would be limited to a band as wide as the greatest interconal distance, embracing the geometrical margin of the image. These successive stimuli would give rise to a concept of a straight line with a position in the center of this zone, this center being the average of the positions of the many cones stimulated. The local sign would, therefore, be accurate to a fraction of a cone diameter, just as the average of many determinations of the position of a point in terms of inches might be accurate to a fraction of an inch.

The experimental evidence is as follows: The length of the image in both the monocular and binocular experiments markedly affected the thresholds, the maximum delicacy of perception being obtained with images equal in length to the diameter of the fovea. Shorter images gave in both cases larger thresholds. This result would agree with the conception of mean local sign in that a reduction of the number of elements involved will give less opportunity for accurate "averaging."

The effect of motion was tested with an apparatus considered to reproduce the essential conditions in the eye, and the recognition of displacement of a line with motion was found to be four to seven times more delicate than without. The analogous case in the eye was tested by reducing the exposure time below the limits of possible eye-movements. In all cases with such short exposures the thresholds were greatly increased.

Monocular and binocular observations of the displaced line were compared and it was found that the monocular threshold was in all cases higher. In those cases where all factors postulated as contributing to the mean local sign were excluded as far as possible, namely, in monocular observation of a short line for less than one-tenth of a second, the thresholds were about five times as large as obtained under usual conditions and irregularities of contour were observed which apparently represent the retinal mosaic.

*Certain features of the physiology of growth as illustrated by the Lamellibranch Tivela.*[1] FRANK W. WEYMOUTH.

The length of the Pismo clam (*Tivela*) for each age, as indicated by the "rings" of the shell which are proved to be annual, has been determined up to 26 years. The course of life-growth expressed in length for this species is particularly accurately represented by these data for the following reasons: Because of the long life the mean length at any age is the average of growth under the environmental conditions of so many different years that the course of life-growth is uninfluenced by the chance succession of favorable and unfavorable seasons. The life includes so many seasonal cycles that the life-growth curve is free from a serious source of confusion present in forms like the insects in which the life-growth and the seasonal growth cannot be distinguished. The disturbances incident to metamorphosis have no appreciable effect. Spawning extends over many years during which the production of eggs gradually increases hence sexual maturity forms no conspicuous epoch, as in fish like the salmon or in man.

The course of life-growth is remarkably uniform and may be very accurately fitted to the formula $y = a - bc^x$. The yearly growths form a decreasing geometrical series in which each term is about $\frac{4}{5}$ of the preceding.

Preliminary examination of the growth of other forms, including mammals, indicates that this type of increase in length (or, with appropriate modifications, in weight) is widespread and probably fundamental. In the more specialized animals there are superposed on this comparatively simple course of growth modifications which may alter its progress. These, like the structural differences with which they are associated, should be considered as special adaptations to the life of the particular species.

We have dealt only with gross total size; the differential growth involved in development will also complicate the picture. The chief occasions of differential growth are the transition from larval to adult life and the onset of sexual maturity. It is apparent that if the course of growth observed in the present case proves to be a fundamental type, an instructive opportunity is offered for the re-analysis of growth in many forms.

*The blood elements in complement deficient guinea pigs.* L. B. NICE, ALMA J. NEILL and H. D. MOORE.

At the Vermont Agricultural Experiment Station, guinea pigs which were deficient in complement[2] were found to be less resistant to disease and more susceptible to changes in temperature than normal animals. This lowered resistance suggested to us that other factors besides complement might be lacking in the blood of these animals. In this study we have determined the number of red and white corpuscles and

[1] Published by permission of The Department of Commercial Fisheries, Fish and Game Commission of California.

[2] Moore, H. D., Journ. Immunol., 1919, iv, 425.

amount of complement and catalase in the blood of 12 complement deficient guinea pigs and an equal number of controls which were kept under the same experimental conditions. Later 10 more normal guinea pigs were tested in the same manner.

A relation between the number of red blood corpuscles and lack of complement was found in our animals. The complement deficient guinea pigs averaged from 37 to 50 per cent fewer red blood corpuscles per cubic millimeter of blood than did the normal animals. This decreased number should lessen the oxygen-carrying power of the blood, and this in turn would decrease the available oxygen in the tissues and may in part account for the lowered resistance of these animals to zero temperature.

The average number of white blood corpuscles was higher in the complement deficient line than in any of the lines of normal animals. This is an indication of a protective device on the part of the organism to make up for the lack of complement as a defense against foreign invasion.

No consistent relationship was found between the number of white and red blood corpuscles and the amount of catalase in the blood.

Two sets of normal guinea pigs fell into two groups as regards the catalase content of the blood; in half of the animals of each set the catalase was low, while in the other half it was more than twice as high. This fifty-fifty ratio in two different sets of normal guinea pigs suggests that high and low catalase may be Mendelian characters.

*A simplified automatic and bloodless method of recording the volume-flow of blood.* ROBERT GESELL.

The method previously described[1] for measuring the volume-flow of blood through the intact vein has been considerably simplified and otherwise improved. One solenoid and one electrical contact automatically stop, propel and record the rate of accumulation of blood in a segment of the exposed vein which in the older device required three solenoids and three electrical contacts.

*Rapid intestinal absorption, without digestion, of a complex protein substance, tissue fibrinogen.* C. A. MILLS.

Tissue fibrinogen, although so complex as scarcely to be able to pass through medium grade filter paper, is rapidly absorbed unchanged from an isolated intestinal loop (dog), producing a prompt accelerating effect on the clotting time of the blood. In these animal experiments the urine was found also to contain an active coagulant, following the intestinal absorption.

In man, very rapid absorption of the material occurs. The clotting time of the blood may be reduced 40 to 50 per cent within $2\frac{1}{2}$ to 3 minutes of the time of swallowing the coagulant. Previous tryptic digestion of the tissue fibrinogen prevents this effect following absorption, as does also inactivation by boiling, so that a digestion and then resynthesis

[1] This Journal, 1919, xlvii, 428.

following absorption are ruled out. The speed of the absorption and distribution of the material necessitates the conclusion that it is absorbed directly into the blood capillaries of the intestinal mucosa. It is entirely too rapid for the lymphatics to have played any part.

The questions involved in this absorption are many. This substance that passes through no semi-permeable lifeless membrane, goes with extreme readiness through intestinal mucosa, capillary walls, kidney epithelium, peritoneum, etc.

*An apparatus for regulating the body temperature of an animal during operative procedures.* MONROE A. MCIVER.

To meet the need of some mechanical device for keeping an animal's body temperature constant throughout the course of an experiment the principle of the ordinary mercury thermostat has been adopted for controlling the amount of external heat applied. The accompanying

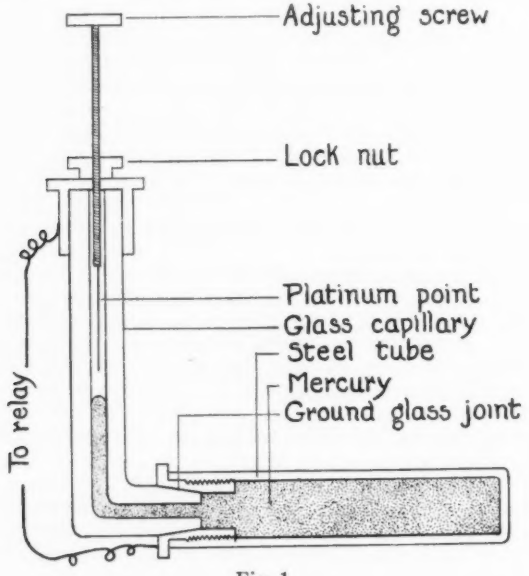



Fig. 1

figure shows an actual-size diagram drawn to scale of the instrument employed. It was designed in collaboration with Doctor Aub and Doctor Redfield.

The structural details of the apparatus are shown in the diagram. It consists of a steel bulb connected by means of a ground glass joint to a capillary glass tube. The upper end of this tube is covered by a metal cap which is fitted with a screw for regulating the height of a

platinum point so that the contact is established at any desired temperature. The wires leading from the thermostat pass to a relay. A one microfarad condenser of the type used on telephones, introduced into the circuit between the thermostat and the relay, has proved of service in greatly reducing sparking when the contact between the mercury and platinum is broken. The relay is connected with the current passing to a heating pad, and is so adjusted that when contact between the mercury and the platinum point is made the current flowing through the heating pad is broken. The instrument when tested in a water bath is shown to be accurate with 0.3°C.

The procedure for use of the apparatus is simple. The anesthetized animal is placed on the electric heating pad. The metal bulb is inserted into the rectum and held in place by a clamp on the upright glass tube. The current flowing through the heating pad is then automatically turned on and off as contact is made and broken in the thermostat.

It is hoped that this instrument may prove useful in physiological work and that with some modification it may be of service in insuring a constant body temperature during general surgical procedures.

*Note on a method of making simultaneous fast and slow drum records of fatigue.* F. M. BALDWIN.

By mounting two aluminum writing rods upon a Harvard-type muscle lever and gradually bending their distal ends through an arc of 90° with each other, it is possible to obtain simultaneous records of fatigue in frog muscle by the fast and slow drum methods. This procedure is of especial value in demonstrations where time or equipment does not permit of each method being employed separately. It has a further advantage in that the two curves supplement each other so as to facilitate the subsequent interpretations as to the duration and amplitude of successive responses as well as relative changes produced by contracture, relaxation, latency, treppe, etc., which obviously are essential to an understanding of such phenomena. The relative speed of each drum may be varied slightly to suit conditions; a ratio of one revolution in 2 seconds for the fast type, to 1 in an hour for the slow type has been found to yield excellent records. Successive stimulations may be regulated by a circuit breaker mounted upon the fast drum housing, and by use of chronometers, the duration intervals of any phase of the process can be calculated and by proper signal devices, any response can be easily located.

*Chemical effects produced by passing electric currents through thin artificial membranes of high electrical resistance.* R. S. LILLIE and S. E. POND.

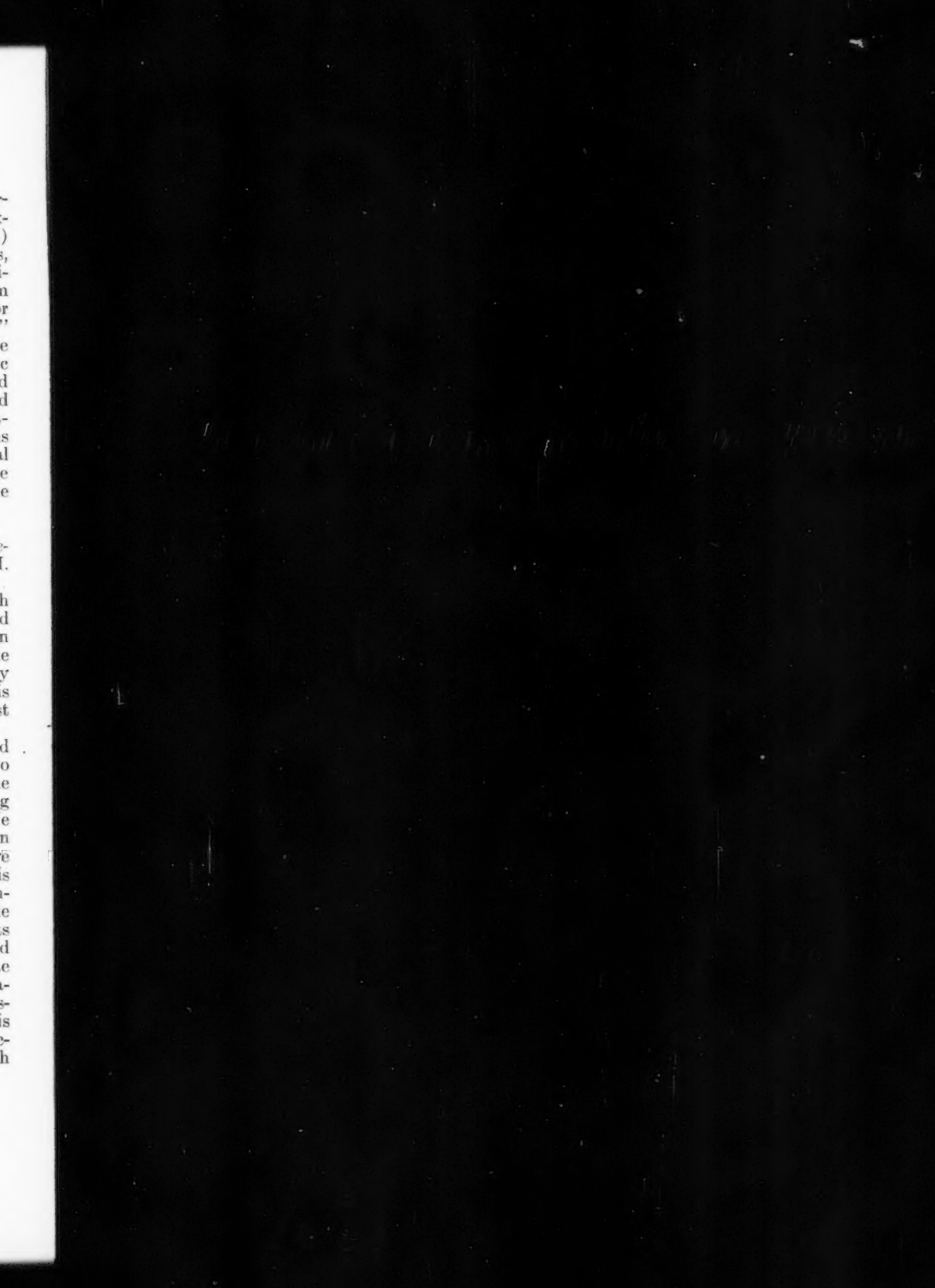
Oxidation of ferrous ($Fe^{++}$) to ferric ($Fe^{+++}$) ion will occur when an electric current is passed through a very thin rubber membrane interposed between two electrolyte solutions one of which is a mixture of ferrous chloride ($FeCl_2$) and potassium thio cyanate (KSCN). The oxidation occurs at the surface of the membrane facing the cathode, i.e., where the positive electrical stream enters the solution from the

membrane. No effect occurs below a certain intensity of electric current, corresponding to a potential of 10 volts across a membrane approximately 50$\mu$ thick. (This is a drop of about 200,000 volts per cm.) Membranes have been made of several substances—viz., water glass, varnish, boiled linseed oil, vanadium linoleate, lecithin-collodion, aluminium oleate, electrolytic basic aluminium oleate, and rubber made from purified gum caoutchouc dissolved in carbon tetrachloride ($CCl_4$) or carbon bi-sulfide ($CS_2$). Supporting fabrics of lens paper, "onion skin" paper, Swiss bolting silk (250 mesh to the inch), or silk chiffon are fastened over the end of a glass tube one inch in diameter. The fabric is then dipped into the solution of the membrane-forming compound and dried. The tube with membrane in place is fitted by a ground joint into a large U-tube and connected through a variable resistance to a source of electric current. Unvulcanized rubber on lens paper gives the best membrane so far constructed. A high electrical resistance (low permeability to ions) is an essential condition for the effect. The work is being continued with other oxidizable and reducible compounds.

*Delay in the response to the second of two stimuli in nerve and in the nerve-muscle preparation.* ALEXANDER FORBES, F. R. GRIFFITH and L.H. RAY.

Lucas observed that when a muscle is stimulated directly or through its nerve a second stimulus applied just after the end of what he termed the "absolute refractory period" evoked a response appearing after an increased interval of time as compared with the interval between the first stimulus and response. A similar observation was reported by Gotch in the case of the electric response of nerve. Lucas ascribed this delayed second response to a modification of the tissue following the first response, such that conduction was slower than normal.

Working with nerve-muscle preparations in both frog and cat we find that in the case of muscle stimulated through its nerve there are two distinct delays involved in the second response, one in setting up of the second nerve impulse by the second stimulus, the other in the setting up of the second muscle response by the second nerve impulse. We find that in a long mammalian nerve there is only a slight decrease in the velocity of the second nerve impulse. Two delays are therefore clearly localized at the point of stimulation and at the junction. This seems to indicate that a modification of the tissue such as Lucas inferred, is not the main cause of the observed delays. We conclude that the time of response in the case of each tissue depends on its refractory phase. In the case of nerve or muscle directly stimulated the delay effect depends on the stimulus falling during the absolute refractory phase and on the well-established fact that the local excitatory process (the essential preliminary to setting up a propagated disturbance) outlasts the stimulus. If the local excitatory process is present the tissue responds when its degree of recovery from the refractory phase is sufficient. The shape of the curve correlating delay with



## ERRATA

In the abstract of the paper by R. S. Lillie and S. E. Pond, page 416, line 3, of the February number, instead of "200,000," read "2000."

the time of second stimulus in the case of nerve corresponds roughly with what we should predict on this view.

To account for the ability of the second nerve impulse to set up a second muscle response after a delayed interval we must postulate some mechanism whereby the second nerve impulse arriving during the absolute refractory phase of the muscle can maintain a source of excitation for the muscle fibers long enough to enable them to respond after their refractory phase. On the membrane theory of the propagated disturbance, which assumes that the action current is the mechanism whereby conduction occurs, the explanation might be found in the fact that the action current in nerve lasts for a considerable time after its maximum intensity; or the effect might also depend on some property in the junctional tissue whereby a still more persistent disturbance outlasts the arrival of a nerve impulse.

Our view that the time of response in each tissue depends on its refractory phase rather than on the retarding modification is still further supported by our observations on the response of the nerve-muscle preparation to three stimuli. The refractory phase following the second (subnormal) disturbance is no greater and probably less than the refractory phase following the first (full-sized) disturbance. The retarding modification should, therefore, be no greater after the second than after the first. In reality the third response in muscle follows the third stimulus after a still greater delay than does the second response. This is simply explained on the view that the time of the third response depends on the ending of the second refractory phase of the muscle.

*The components of the action currents obtained from nerves.* H. S. Gasser and Joseph Erlanger.

The action current in cold- and warm-blooded nerve studied by means of an amplifier working into a cathode ray oscillograph often exhibits catacrotic waves.[1] Study of these waves indicates that they are discrete action currents originating simultaneously with the main action current, but travelling at slower rates, presumably in fibers possessing different physiological characteristics.

That they travel at different rates is proved by comparing the contours of records of the action current at different stages in its progress along the nerve. Calculations based on such records give, by way of example, the following propagation rates: bull frog's sciatic at 22.3°C., primary or $\alpha$ wave 42, $\beta$ wave 23, $\gamma$ wave 14 meters per second; dog's saphenous at 38°C., $\alpha$ wave 80 and $\beta$ wave 46 M. p. s. The duration of the stimulating induction shock being known to be less than one-third of the measured refractory phase in cold-blooded nerve the waves could not possibly owe their origin to a "repetitive" process started by a prolonged stimulating current. That they could be repeated waves at all may be negatived by the fact that all the waves seem to start simultaneously. With leads in a constant position the height of the action

[1] Gasser and Erlanger: This Journal, 1922, lxii, 496.

current decreases with the distance traveled. This can easily be explained on the theory of constituent waves of different characteristics but not on the supposition that an extra process is started in the individual fibers.

Though the waves are brought out best by strong stimulation, they may be present when the stimuli are just maximal. They are not, therefore, due to the use of strong stimulation per se. Additional proof of this consists in the fact that in the action current of the phrenic nerve waves are never present at any strength of stimulation whatever. The differences in conduction rates of the waves ($\alpha$, $\beta$, $\gamma$) are not due to the development of discrete regular decremental states resulting from injury to the nerve in preparation. For if this were the case waves should appear in the phrenic as in other nerves. As a matter of fact, injuring the phrenic nerve by manipulating it roughly, or by painting it with scalding water or with 95 per cent alcohol, does not cause waves to appear in its action current.

The composition of the action current seems to be more or less characteristic for each nerve, thus the action current in the tibial, the saphenous, the vagus and the phrenic nerves from the same animal have distinctive forms which are fairly constant for each of the pairs.

*Rapid intestinal absorption, without digestion, of an unaltered complex protein substance, tissue fibrinogen.* C. A. MILLS.

Tissue fibrinogen may be taken by mouth and within $2\frac{1}{2}$ to 5 minutes cause a marked shortening in the blood clotting time. Since it is readily inactivated by acid, alkali or proteolytic enzymes, it must be given when no digestion is in progress. Given with ice water to dilute any juices present and stimulate peristalsis, it rapidly enters the intestine and is absorbed unchanged. Before breakfast the results are most prompt, but the effects last longer at other periods of the day. As high as 50 per cent shortening of the clotting time has been observed within $2\frac{1}{2}$ minutes after swallowing the coagulant. Hemophiliacs show the same relative shortening as do normal individuals.

No such effects are seen if the coagulant be digested by trypsin or boiled before swallowing, so a digestion and resynthesis is ruled out.

Kidney excretion of the active coagulant has been observed in animals.

The speed of the reaction necessitates the conclusion that there is direct and rapid absorption into the blood capillaries. The lymphatics could play no part in so rapid a process.

Vaccines, antitoxins, antisera, pituitrin and insulin are now being tried after the above fashion.

*The excitation of gastric secretion by application of substances to the duodenal and jejunal mucosa.* A. C. IVY and G. B. MCILVAIN.

Our experiments were performed on dogs with a Pavlov pouch and a Thiry fistula of the duodenum and the first portion of the jejunum. The Thiry fistula was made by transecting the duodenum 1 inch below

the opening of the duct of Santorini and the jejunum approximately 4 inches caudal to the duodeno-jejunal juncture. The distal end of the intestinal loop was closed and the other end brought to the exterior via a stab wound opening in the abdominal wall. Then an end-to-end anastomosis was done between the duodenum and the jejunum. This produced a loop of duodenum and jejunum approximately 15 inches long, so that substances could be continuously applied to its mucosa and the resulting effect upon the secretion of the Pavlov pouch observed. Nine dogs have been prepared and kept alive for 1 month to 7 months in excellent health.

The following experimental procedure was followed: *1*, Two 1-hour collections of the continuous secretion of the Pavlov pouch; *2*, application of the substance to the duodenum for 15 to 60 minutes; *3*, 3 hours' collection after the application; *4*, injection of "gastrin" or feeding a meal to demonstrate the physiological response of the Pavlov pouch to known excitants.

The following substances have been found to be excitants of the secretion of the Pavlov pouch when applied to the duodenum: N/80–N/10 HCl, 5 per cent soap solution, N/2–N/10 butyric acid, N/20 HCl in olive oil emulsion, 5 per cent and 10 per cent glucose, 10 per cent magnesium sulphate, 10 per cent NaCl, gastric juice (dog), 10 per cent glycerine, 7 per cent Armour's meat extract and 10 per cent peptone (Witte's).

The following substances have proved to be negative up to the present time: fresh meat juice, ground meat (200 gm.) digested 24 to 48 hours with 400 cc. of gastric juice (no free acid present in digested mixture), olive oil, 0.3 per cent and 1.0 per cent sodium carbonate, 0.5 per cent mustard oil, N/10 ammonium hydroxide, extract of smoked highly flavored sausage, 1 per cent barium chloride, distilled water, 0.6 per cent and 0.9 per cent NaCl.

N/10 HCl and 20 per cent ethyl alcohol are maximal excitants. The Pavlov pouch does not always respond to the excitant, the occurrence of the response and the degree to which it occurs depending apparently on the secretory tone of the gastric glands.

Excitation also occurs if a Heidenhain pouch dog is used. N/20 HCl put in the duodenum via duodenal tube in two men caused stimulation of gastric secretion.

The latent period for the excitation of gastric secretion from the duodenum is 20 to 30 minutes in the case of glucose and alcohol and approximately 45 minutes in the case of HCl.

The acid washings of the duodenum, extracted by the Koch method for gastrin, excites, when injected intramuscularly, gastric secretion in a Pavlov pouch dog.

Our present observations only prove that it is possible to excite gastric secretion by application of the solution of various substances to the mucosa of the duodenum and the first part of the jejunum.

*The behavior of the empty stomach in the mollusca.* T. L. PATTERSON.

As yet there are insufficient data to show the differences and similarities between the types of gastric activity in the various animal groups and this is notably true in the case of the invertebrates. The present investigation is limited to two species of marine mollusca, which, insofar as I am aware, represents the first study to be made on the movements of the empty stomach in any of the invertebrates. The first is a large belly-footed animal or univalved shell-fish known as Haliotis rufescens (Abalone), while the second is a large chiton called Cryptochiton stelleri, both species of which are found on the Californian coast. The contractions were recorded by the balloon method, the animals being kept in a large vivarium provided with running sea-water.

In Haliotis an esophageal fistula was made about 5 cm. posterior to the head and mouth by simply raising the shell and making a small transverse incision laterally with scissors through the exposed wall of the esophagus. The balloon was introduced through this opening and pushed into the stomach and the rubber tube for the manometer then carried through one of the fissures (openings) in the shell. The contractions always started in 12 to 15 minutes following this procedure and were continuous. However, a certain type of altered periodicity was noted as indicated by periods of activity with the strong hunger contractions separated by comparatively long intervals of rest without cessation of movement. In starvation, the gastric contractions progressively increased in strength until after about 2 weeks, when they approached the form of incomplete tetanus. The introduction of sea-water, fresh tap water, 1 per cent sodium carbonate or 0.5 per cent hydrochloric acid directly into the stomach produced inhibition in varying degrees, the intensity being indicated in the order of the respective substances given, it being the least in the case of the sea-water.

In Cryptochiton, a fistula was found to be impracticable and it became necessary to use a glass stomato-gastric tube covered with the rubber tubing which fitted into the mouth and passed into the much folded stomach with the balloon attached to the end. The contractions of this animal were also continuous with only a slight indication of an altered periodicity. They appeared to be analogous with the twenty-second rhythm in man.

External mechanical and chemical stimuli when applied to sensory surfaces of either of these animals as the mantle, epipodia, sense palps, etc., invariably produced temporary inhibition of the gastric hunger movements but its duration did not usually exceed greatly the duration of the stimulation unless severe.

A histological study of the alimentary tube of Cryptochiton which is very thin revealed the absence of the longitudinal muscular layer, a very thin circular muscular layer especially in the cardiac end of the stomach, a somewhat thickened basement membrane supporting columnar-shaped epithelial cells containing probably cilia. A similar study of the alimentary tract of Haliotis revealed its structure to be similar to that found in the alimentary canals of higher animals.

*The motile state of the stomach of the monkey in hunger.* T. L. PATTERSON, JESSIE ILLENDEN, L. W. RUBRIGHT and R. J. SCOTT.

Studies were made on the movements of the empty stomach of the Javanese monkey (Macacus sinicus) 18 to 24 hours after feeding. Gastric fistulas were made on these animals after thorough taming, the operative procedure being similar to that used by Carlson on dogs.[1] The contractions were recorded by the balloon method, the animals being held quietly in the lap in a room free from disturbing influences. The contractions exhibited a definite periodicity similar to that found in the higher animals with the exception that both the periods of activity and of quiescence appeared to be of considerable shorter duration as was shown in young adult animals. The periods of hunger activity usually ended in strong tetanus with a marked increase in gastric tonus which rapidly fell at the termination of the period. Otherwise the contractions of the monkey's stomach were practically identical with those of man. During the quiescence periods the animals were more at ease and at times would even doze off to sleep, while the periods of strong hunger activity aroused and caused them to become restless. The introduction of water, 1 per cent sodium carbonate or 0.5 per cent hydrochloric acid directly into the stomach invariably produced inhibition, the degree of the intensity being indicated in the order of the respective substances given, it being the least in the case of the water.

*The effect of formaldehyde upon the vitamin content of milk.* A. M. BLEILE and R. J. SEYMOUR.

The utility of chicks and the advantages of using these animals in vitamin tests was pointed out by Seymour and Durrant in 1919, and their findings have recently been confirmed by Emmett and Peacock in a series of tests in which comparatively large numbers of chicks were used. Baby chicks are particularly susceptible to a lack of vitamins and thus seemed especially suitable to determine the possible effect of formaldehyde upon the vitamins of milk. Chicks were fed vitamin-free food to which milk was added to make a barely moistened mash. Raw milk served as the sole source of liquid during the course of the experiment. One-half the chicks were given raw milk to which had been added sufficient formaldehyde (1:20,000) to prevent souring at room temperature for 48 hours. As previous investigations upon the effects of formaldehyde, particularly those by Price upon calves, had shown that formaldehyde in small quantities seemed to be entirely inert, the question arose of its possible effect upon vitamins. The fact that "winter" milk (supposedly lower in vitamins) was used, coupled with the great susceptibility of chicks to lack of vitamins, would apparently show whether formaldehyde exerts any deleterious effect upon the vitamin content. In the tests carried out in March and November no noticeable differences were found between the control chicks and those using the treated milk. Growth curves were almost identical, such difference as occurred being in favor of the chicks receiving formal-

[1] Carlson: This Journal, 1913, xxxii, 369.

dehyde; xerophthalmia and polyneuritis appeared in each group toward the end of the tests, but in each case appeared in the control chicks at an earlier period.

*Study of pulse wave velocity in arterio-sclerosis.* JANE SANDS.

The present study is concerned with the measurement of pulse wave velocity in arterio-sclerosis. Various clinical types were chosen mainly those having either a high blood pressure or those presenting thickening as determined by palpation.

The method used was that of taking simultaneous records of apex beat or heart sounds, brachial or femoral, and radial or dorsalis pedis, along with an electrocardiogram. The tracings were taken using Wiggers' modification of Frank's capsules. The tracings were read as accurately as possible sometimes using a magnifying lens and sometimes a Keith Lucas comparator. Corrections were made for parallax and in comparing the electrocardiogram with the pulse tracings a correction was made for transmission time. It was not always possible to read the apex beat record accurately, therefore control readings were made from the peak of "R" allowing a standard correction of 0.06 second for rising tension time.

Tracings from sclerosed vessels show definite and, in this series of cases, constant variations in pulse wave velocity from the normal, if the figures given by Bazett and Dreyer are taken as standard. In the larger vessels the pulse wave velocity is increased, the rate being two to three times more rapid than the normal. In the peripheral vessels, however, the velocity is decreased, contrary to expectation. The degree of variation seems to be in direct proportion to the amount of thickening in the vessels, as determined by palpation, and there was a very slow transmission time from brachial to radial even when the vessel was distinctly nodular.

*The electrocardiogram in fasting dogs.* HOMER C. LAWSON, S. MORGULIS and A. E. GUENTHER.

Experiments were undertaken upon five dogs to determine the nature of the cardiac reponse resulting from starvation. Electrocardiograms were taken at 3- and 4-day intervals, before, during and after fasting. The data from two dogs are presented. However, four dogs showed a similar trend in regard to percentage loss of weight, changes in heart and respiration rates, and in the arrythmia of the heart. During a preliminary period the dogs were fed to a constant weight and then food was entirely withheld but plenty of water supplied, until the animals had lost 40 per cent of their weight. After this they were again fed. During recuperation the cardiac recovery was quite disproportional to the slower recovery in body weight.

Data concerning the progress of the fasting effects on dog 2 are given in table 1. The letters *A*, *B*, *C*, *D* and *E* refer to a section of five electrocardiograms from the twelve taken. In taking the records the experimental conditions were alike insofar as they were under control.

Apart from the general behavior of the animal the only guide to its normalcy was the presence of the anticipated rectal temperature.

The records of dog 2 showed at the outset premature beats and a left preponderance. During the fasting period the preponderance disappeared completely, only to reappear to a greater degree with recuperation. The T-wave, indefinite before fasting, was strikingly negative when the weight loss reached 14 per cent and became strikingly positive when the weight loss was 40 per cent. It was pronouncedly negative again on the 15th day after the termination of the fast. The form of the P-wave remained fairly constant throughout the records.

TABLE 1

| | PERIODS | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before | During fasting | | | | | | | | | After | |
| | 1 day | 3 days | 7 days | 10 days | 14 days | 17 days | 21 days | 24 days | 31 days | 33 days | 1 day | 15 days |
| Per cent body loss | | 5.6 | 12 | 14 | 20.5 | 21.9 | 27.1 | 29.0 | 35.8 | 40 | | |
| Respiration per minute | 58 | 20 | 20 | 18 | 15 | 18 | 24 | 24 | 20 | 24 | 60 | 30 |
| Heart rate per minute | 87 | 99 | 82 | 67 | 68 | 44 | 45 | 54 | 65 | 69 | 153 | 117 |
| Electrocardiogram | A | | | B | | | | | | C | D | E |

Dog 1 exhibited during the fasting period similar features. The progressive loss of weight was accompanied by a cardiac slowing and arrythmia both of which disappeared toward the end of the fast. The administration of the first meal following the fast resulted in an acceleration of the heart beat from 87 to 133 per minute. At no time did this dog show any preponderance, but the T-wave manifested the same instability as shown by dog 2.

*Relation of the ovary to the gravid uterus in the aplacental opossum.* CARL HARTMAN.

In the higher mammals and man the internal secretion of the ovary (corpus luteum) is necessary for the implantation of the embryo, i.e., for placentation (Leo Loeb). It appears, however, that the ovarian hormone influences the entire uterus, including the musculature, as indicated by Ancel and Bouin's observations on pseudopregnant rabbits after sterile coitus.

This matter was tested on *pregnant* as well as pseudopregnant females of an aplacental species, the opossum. Removal of both ovaries in this form invariably results in the death of the embryos in the course of 3 days or more, with subsequent absorption in utero. In pregnancy or pseudopregnancy the operation always causes excessively rapid involution of the uterus, which returns to the anoestrous or juvenile condition.

By way of control it is found that excision of uterus and ovary on one side or of one uterus and the contralateral ovary has no effect upon the progress of gestation in the surviving uterus.

It is evident, therefore, that ovarian secretion does not merely sensitize the uterine mucosa for the formation of the decidua but that its influence is more profound. This applies certainly to the opossum, in which there is no decidua, and probably to the Placentalia also.

*Administration of lactic acid following removal of the liver.* FRANK C. MANN and THOMAS B. MAGATH.

Previous experiments have proved that *1*, total removal of the liver is always followed by a decrease in blood sugar; *2*, the hypoglycemia is associated with definite and characteristic symptoms; *3*, administration of glucose prevents the decrease in blood sugar and the development of these symptoms; and *4*, the glucose thus administered is used by the organism.

If lactic acid is an intermediate product of carbohydrate metabolism and can be used as such from an extracellular source it might be possible to substitute lactic acid for glucose in hepatectomized animals. Both racemic and d-lactic acid were administered in suitable amounts, either intravenously or through a jejunostomy, in the acid form or neutralized, to a series of hepatectomized animals. In no instance was the decrease in blood sugar affected nor did lactic acid benefit the animals when they had developed the symptoms of hypoglycemia. These experiments suggest that if lactic acid is an intermediate product of glucose metabolism, it can only be utilized intracellularly and must enter the cell in some other form.

*The influence of the cerebrum and cerebellum on decerebrate and ether rigidity.* J. M. OLMSTED and W. P. WARNER.

Extensor rigidity in the hind and front legs of cats was produced by ablation of the contralateral cerebral cortex at a high level. Ablation of the cortical motor area alone did not produce rigidity, ablation of the frontal cortical area produced contralateral rigidity.

Unipolar stimulation of the cut surface of this frontal area abolished contralateral rigidity. Stimulation of the cut cerebral cortex slightly posterior, i.e., on the medial side of the internal capsule also abolished rigidity. The stimulation of the other fiber of the internal capsule gave contralateral movements, sometimes flexion, sometimes extension.

A tract carrying fibers which inhibit contralateral decerebrate rigidity was thus found apparently extending from the frontal area to the internal capsule. Extensor rigidity produced by light ether anesthesia was also used to locate this inhibitory tract. Stimulation of the frontal area caused the ether rigidity on the contralateral side to disappear.

This inhibitory effect produced upon and stimulating the control tract was abolished by cutting the contralateral middle cerebellar peduncle. Contralateral rigidity was produced by cutting this peduncle and abolished by stimulating it.

*The distribution of the vitamin A in the urine and the digestive secretions (man, dog).* ETHEL COOPER.

The urine and digestive secretions were concentrated, extracted with ether and alcohol, and these extracts added to a standard rat diet, adequate for growth and maintenance, except for vitamin A. The young and growing rats were first put on the standard diet for a period of 10 to 14 days, to test their susceptibility to vitamin A deficiency. The ether-alcohol extracts of the body fluids were added to the standard diet in the proportion of 2 cc. of urine or digestive secretion to 1 gram of diet.

The urine of dogs on ordinary laboratory diet (table scraps) contains little or no vitamin A. When cod liver oil, eggs and milk are added to the diet, much of the vitamin A appears in the urine. The urine of man on an ordinary diet (including one pint of milk per day) contains little or no vitamin A. When cod liver oil, milk and eggs are added to the diet much of the vitamin A appears in the urine. The urine of fasting men (15 days' fast) contains no vitamin A.

Human saliva, even on a vitamin A rich diet, contains little or no vitamin A.

Dogs' gastric juice (Pavlov pouch) contains considerable vitamin A only when the diet is rich in this substance. Human gastric juice (appetite secretion) contains little vitamin A on diets including eggs and milk. The continuous secretion contains little or no vitamin A. The gastric jiuce for these tests was secured by Doctor Carlson from a child of 12 years of age (gastric fistula and stenosis of the esophagus). Human pancreatic juice (pancreatic fistula) tested 9 months after collection contained no vitamin A.

Both the ether and alcohol extracts of bile (ox) when added to the diet of the rats in the proportion of 2 cc. of bile to 1 gram of diet proved highly toxic. The toxic agent does not appear to be taurocholic acid. Because of the high toxicity of the bile extracts the question of vitamin A in the bile could not be settled.

The urine and digestive secretions showing little or no vitamin A by these tests may nevertheless contain this substance but in too small concentrations for the quantities of extracts fed. Some of the vitamin is also probably lost in the concentration and extraction of the secretions.

The vitamin, if sufficiently concentrated in the blood, passed into most if not all of the body secretions, including the urine. The alimentary vitaminuria on high vitamin A diets probably indicates a limited capacity of the organism to store or destroy vitamin A.

*The effect of voluntary contractions and of dermography on the number of visible capillaries of the human skin.* TSANG G. NI.

The number of patent capillaries of the human skin of different parts of the body, and of the same skin region under various influences is changeable. In the human skin only those capillaries which are opened up and contain red corpuscles can be rendered visible. To count the visible capillaries of the normal skin, therefore, may serve to

study some factors affecting the blood circulation in the capillary area. A binocular microscope and a Whipple eyepiece micrometer were employed for such studies.

Voluntary isotonic contractions of a group of muscles of a hand caused an initial decrease and then an increase in visible capillaries of the local region. Both the initial fall and the secondary rise were slight. The voluntary isometric contraction gave a high "capillary counting" immediately after as well as during the contraction. During the isometric contraction the increase of patent capillaries of local areas was more than twice.

Experiments were made to see whether there were visible changes in the capillaries, which would account for the dermographic reaction, as many writers have adopted the view that it was a capillary phenomenon. In most observations "capillary countings" were made in connection with the red line, which could be more clearly seen than the white line. Control countings were usually performed before any application of stroke.

In one series of experiments capillaries were counted before and 30 seconds after the application of the stroke, the red line being usually at its height in from 30 to 50 seconds after the stimulation. There was either a slight increase or a decrease in the number of patent capillaries in the red line. In another series, the number as well as the size of capillaries was noticed and compared. It was observed that the increase in the number of dilated capillaries in the red line was slight or none. By actually observing the condition of the capillaries and by counting them, the result would seem to show that the dermographic red line is not due simply to a dilatation of the capillaries of the skin.

*The silver non-polarizable electrode adapted for use in electrocardiography.* MELVIN DRESBACH.

To provide a simple and convenient type of electrode for electrocardiographic work various metals, brought into contact with the animal body by means of sodium chloride soluton, have been used. The physical contact of sodium chloride solution with the metals ordinarily used, viz., lead, copper, zinc, galvanized iron, German silver, does not form a non-polarizable junction, though in practice the amount of polarization is small and often negligible. The silver electrode, long ago suggested in principle by D'Arsonval, and in recent years used by Lapicque, Lucas and Adrian, lends itself admirably to electrocardiography. A thin sheet of pure silver, which need not be over $\frac{1}{32}$ inch in thickness, is cut so as to provide three or more pieces, $1 \times 3$ inches, each piece having a narrower portion $\frac{3}{8}$ inch wide extending $2\frac{1}{2}$ or 3 inches from one end. To the end of the narrow part a binding post is attached. The silver plate is then coated with silver chloride. To do this, the perfectly clean plate is connected to the positive pole of a low voltage (2–6) battery; to the negative pole a piece of platinum foil is connected. The two metals are then dipped into a sodium chloride solution for about 2 minutes. The silver becomes firmly coated with silver chloride, which undergoes reduction, a matter of no importance here.

Theoretically, as long as there is enough silver chloride on the surface of the plate to saturate the water in contact with it, the electrode is non-polarizable with currents of low amperage. The form and dimensions given above are arbitrary and permit it to be bound onto the arm or leg, even of an infant, by means of a bandage wet with 20 per cent sodium chloride warm as can be borne comfortably. It is important, however, to bring the bandage into contact with a large area of the skin, for example, of the entire forearm or leg. This is necessary in using any metal, whereas the surface area of the metal itself can be relatively small, the resistance of the metal-bandage combination being only about 0.5 ohm.

Such a silver electrode can be used for several weeks without recoating providing it is not roughly handled or soiled. It is non-polarizable; does not deteriorate; it not easily broken and is relatively cheap.

*The site of the anaphylactic reaction of the uterus.* O. O. STOLAND.

The response of the uteri from guinea pigs sensitized to egg white and dog serum was studied in vitro. When the response of one horn indicated sensitization to the antigen, the other horn was treated with 0.25 to 2 cc. of 1 per cent atropin sulphate in 25 cc. Tyrode's solution. The smaller doses of atropin produced an increased tone in the uterine muscle but the larger doses had no effect on tone or produced an inhibition. Doses of antigen which caused a marked contraction of one uterine horn (non-atropinized) from a sensitized guinea pig, failed to produce a contraction in the other horn following treatment with the different doses of atropin. The failure to respond was not due to paralysis of the muscle by atropin since they never failed to respond to pituitrin administered following the antigen treatment or after another treatment with atropin.

The atropin failed in some cases to prevent the response of non-sensitized uteri to toxic doses of protein indicating that the toxic action of protein is upon a different part of the mechanism than the antigen.

It is possible that the atropin paralyzes the nerve endings or that part of the peripheral nervous mechanism upon which the antigen acts. Since atropin abolishes the pilocarpine action of uteri in vitro it would seem that the antigen affects the same part of the mechanism as pilocarpine.

Another possible explanation of our results that may be suggested is that the muscle is de-sensitized to the antigen by the atropin but retains its irritability to other uterine muscle stimulants.

*Causation of the differential blood pressure in aortic regurgitation.* H. C. BAZETT.

The differences observed in blood pressure between the arm and the leg have usually been ascribed to some contraction in the femoral vessel. In arranging experiments for class work with a circulation schema it was discovered that a differential blood pressure in favor of the femoral

could be produced in a circulatory schema quite easily and was of similar magnitude to that observed in animals, and did not depend on the character of the vessel wall.

The method used was simply to represent the circulatory system by rubber tubing with different branches, the connections to branches being made with short lengths of metal tubing. The arteriole was represented by glass tubing of considerably larger bore packed with cotton. Water was run into this system from a large reservoir slung at a considerable height above it, the flow being interrupted at intervals by a rotating tap designed by Doctor Reichert. This tap has an opening by which the arterial system can be allowed to have a back flow during the diastolic period. It was found that in such a schema normally the systolic pressure record in the femoral was slightly above and the diastolic slightly below that recorded in the schematic carotid and that this slight difference was very much exaggerated in conditions of aortic regurgitation. Under such circumstances the systolic pressures in the two vessels might both be above that of the available pressure from the reservoir, but the exaggeration of the pressure was much more marked in the schematic femoral.

Experiments were done varying the length and character of the tubing in different ways and it seemed definite that in such a schema the difference between the carotid and the femoral was determined by the mass of fluid contained in the tubes between the schema tap and the recording point. If the cause is the same for man then the conditions in the femoral artery would be due to the momentum of the large mass of fluid contained in the aorta under conditions of rapid changes of velocity such as occur in aortic regurgitation. The only discrepancy between the schema and the figures given for aortic regurgitation in man is that most observers have given figures suggesting that the diastolic pressure is also raised in the femoral in such cases. But the accuracy of such figures in limbs which are often very muscular seems to me to be doubtful, especially as some clinical observers have found the reverse, namely, lowering of the diastolic pressure, so that the discrepancy is not serious.

*Experiments on the migration of bacteria.* SHIELDS WARREN and STUART MUDD.

In a series of experiments to determine the comparative motilities of Vibrio percolans and V. cholerae asiaticae,[1] employing the method of Carnot and Garnier,[2] the migration of the motile organisms was apparently determined by the available food supply.

The apparatus used was a glass U-tube of 14 mm. inside diameter, with arms 20 cm. long, having a plug of glass wool placed in the bottom of one arm. Above this was placed a layer of 60/120 quartz sand (average diameter of grains 0.23 mm.) about 10 cm. in depth. Hay infusion or nutrient bouillon is poured in, filling both arms to a level

[1] Mudd and Warren: Journ. of Bacteriol. In press.
[2] Besson: Technique Microbiologique et Sérothérapeutique. Paris, 1914, 149.

1 cm. above the sand. The arms are plugged with cotton and the whole autoclaved.

When the small amount of medium above the sand was inoculated, both V. cholerae and V. percolans worked through the sand layer and clouded the clear arm in 72 hours or less. On the other hand, when the clear arm was inoculated, the medium above the sand did not become clouded, though some of the tubes were kept 23 days, when the medium had evaporated to the level of the sand.

The experiments were repeated with sterile Berkefeld filtered fluid from an 8-day culture of V. percolans in hay infusion, of very low nutritive value, substituted for the usual medium in the U-tube. Inoculation was effected by placing 2 cc. of 24-hour hay infusion culture of V. percolans above the sand. In none of the four tubes used did the organisms grow through into the clear arm until the 9th day, while they did in 60 hours in the control tubes of fresh hay infusion.

In four tubes filled with hay infusion made by soaking fresh hay in the original lot of "used" infusion, and inoculated above the sand layer, the organisms grew through in 84 hours or less. From this last experiment it would appear the the failure of the organisms to grow into the "used" medium is due to its lack of nutritive value and not the presence of inhibitory substances.

In other experiments it was found that highly motile organisms could be selected by inoculating the medium above the sand layer, and then as soon as the clear arm became clouded, inoculating from this a fresh tube above the sand. During six to eight transfers the time of passage through the sand steadily decreased and then reached a constant point. Thus V. percolans in the first tube averaged $7\frac{1}{4}$ hours per centimeter of sand, and at the end of several transfers worked through in about $2\frac{1}{2}$ hours per centimeter, which rate could be maintained so long as transfers were made rapidly. V. cholerae asiaticae, with an initial time of $5\frac{1}{2}$ hours per centimeter, reached a maximum of $1\frac{3}{4}$ hours per centimeter.

*Certain factors affecting filtration through Berkefeld candles.* STUART MUDD.

A readily cultivable spiral organism, Vibrio percolans (new species), has been isolated from fresh water hay infusion. V. percolans passes readily through Berkefeld V but not N candles. Air can be forced through wet N and V filters at the same pressures; the greater permeability of V candles seems therefore due to their possessing more gross pores or lacunae rather than to larger size of the ultimate pores.

When motility of V. percolans is stopped with acid, i.e., $H^{\cdot}$ greater than $5 \times 10^{-6}$, with narcotics or cold, filterability through V candles is also suppressed.

The contact surface of the pores of a Berkefeld filter and the fluid bathing them is the site of an electrical potential difference, an ordinary Helmholtz double layer, the solid walls carrying a negative and the liquid a positive charge. We would predict, therefore, that when solutions or

suspensions are filtered, positively charged particles, at least to some extent, would be absorbed on the filter walls and thus removed from solution, while negatively charged particles would be borne through by the filtration stream. This expectation we find fulfilled with dyes. Solutions of "basic" dyes like methylene blue, in which the colored ion is electropositive, may be completely decolorized by the filter at first, only the later portions of the filtrate becoming colored by the dye. Solutions of "acid" dyes like eosine in which the colored ion is electronegative, pass through a clean Berkefeld candle without demonstrable change.

These observations become of biological interest because of the fact that proteins and immune bodies and enzymes, or at least the proteins with which they are intimately associated, are amphoteric, possessing properties analogous in acid solution to those of methylene blue, in alkaline solution to those of eosine. More exactly stated, a protein at hydrogen ion concentrations higher than that of its isoelectric point dissociates predominantly as a cation, in alkaline solution as an anion. Until proven to the contrary, therefore, protein solutions in small amounts which it is desired to get through into the filtrate would be more safely filtered on the alkaline side of the isoelectric point of the protein in question.

By causing a potential gradient between electrodes within and without a Berkefeld filter fluid may be drawn rapidly through the filter to the negative electrode side. Preliminary experiments indicate the possibility at least that this electroendosmotic streaming can bear vibrios through the V candles.

*On the concentration of proteins in tissues.* EDWIN J. COHN.

Invertebrate tissues are much less firm; contain more water and fewer solids than do the tissues of fish, amphibians or reptiles, and these in turn than those of mammals. Associated with this increase in the packing of solids in tissues there is not only a decrease in water, but also in electrolytes. The freezing point lowering of the blood of marine invertebrates is almost exactly equal to that of sea water (2.3°C.); while the freezing point lowering of the blood of teleost fishes is less than half (1.04 Charax puntazzo to 0.74 Crenilabrus pavo); that of amphibians and reptiles still lower; and that of mammals about a quarter (0.526 man, 0.619 sheep) of that of invertebrates, or of the seawater.

That the concentration of such dissociable, non-diffusible contents of cells as the proteins must lead not only to the loss of water, but also to the loss of the diffusible electrolytes with which the proteins are in equilibrium, follows from the important studies of Donnan and of Loeb upon membrane equilibria. Osmotic work, however, would have to be performed in order to effect and to maintain such concentration, and a difference in osmotic pressure would result. In such systems this difference in osmotic pressure could again be eliminated by affecting the distribution across the membrane of the non-diffusible as well as of the diffusible electrolytes, since when the concentrations of such non-

diffusible electrolytes as proteins are equivalent on both sides of a membrane, equable distribution of the diffusible electrolytes follows, and osmotic equilibrium is reëstablished.

Observations made in connection with an investigation of the physical chemistry of the serum proteins suggest that their concentration in blood is approximately equivalent to that of the proteins in the cells with which the blood is in equilibrium. The red blood cell is the most available for study of those in equilibrium with plasma. When the hemoglobin that it contains was studied by methods similar to those employed with the serum proteins it was found that, at the reaction of the blood, hemoglobin bound approximately as much base as the combined serum proteins. At the same hydrogen ion concentration, oxyhemoglobin binds more base than reduced hemoglobin. This difference in the amount of base bound results in a disturbance of osmotic equilibrium, and the passage of water. This, and many other disturbances of osmotic equilibrium unquestionably occur. None the less the coincidence above noted must be considered an important factor, and give rise to new conceptions and hypotheses concerning the heterogeneous equilibria that obtain in the body.

*Some effects of adrenalin on the perfused bullfrog heart.* C. I. Reed.

An extensive series of records was taken on the denervated heart using a simple perfusion system with dilutions of adrenalin ranging from 1:10,000 to 1:300,000, also a series of control experiments perfusing with Ringer solution. The effects on force, rate and tonus were recorded. The minute rate multiplied by the amplitude as measured in millimeters on the graphs was used as an efficiency index.

It was noted that the auricle was less sensitive than the ventricle, that there was great variability in response, possibly a species peculiarity, that this heart has a higher threshold than that of mammals, 1:300,000 being the greatest dilution producing any response. There was also a wide range of sensitivity, heart rate being affected much less frequently than force but the most common effect was one of increased efficiency, for a limited period, after which there was either a gradually developed tolerance so that the heart returned to normal, or, less frequently, a toxic arrhythmia from which the heart did not recover. Occasionally sensitization occurred after one application. Where toxicity was not induced the most pronounced effect was always early in the experiment.

Owing to the fact that atropine did not alter to any extent the degree or character of reponse it is apparent that adrenalin, in this species, acts largely on the musculature itself.

*The effect of gymnasium exercises and athletic contests on the blood catalase.* W. E. Burge.

Subjects for the experiments were university students. The gymnasium exercises consisted of wrestling, weight lifting, calisthenics and the athletic contests, football and cross country running. Blood

catalase was determined before and after the exercises. The determinations were made by adding 0.5 cc. of blood diluted one to three with 0.9 per cent sodium chloride, to 200 cc. of neutral hydrogen peroxide in a bottle, and the amount of oxygen liberated in 10 minutes was taken as a measure of the catalase content of the blood. Determinations of the weight of the dried blood, and red blood cell counts were made before and after the exercise.

Weight lifting for 15 minutes increased the amount of oxygen liberated by the blood from 876 cc. to 1130 cc., wrestling for 20 minutes from 820 cc. to 930 cc., dumb-bell drill for twenty minutes from 1170 cc. to 1275 cc., and cross county running from 980 cc. to 1165 cc.

The effect of football playing on the blood catalase was studied during the whole football season, which extended from the first of September until the last of November. The first game of the season increased the amount of oxygen liberated by the blood of one of the line men from 780 cc. to 1000 cc., and of the quarterback from 770 cc. to 990 cc.

During the season the normal catalase content of the blood gradually rose, so that at the end of the season it was about 25 per cent higher than at the beginning. The rise produced by a game was less at the end of the season than it was at the beginning.

The increase in oxidation brought about by exercise is attributed to the increase in catalase. The seasonal rise in catalase during a period of training such as occurs during a football season is interpreted to mean that the oxidative processes had been increased to a higher level.

*The effect of food, starvation and low temperature on the catalase content of paramecia.* W. E. Burge.

Catalase determinations were made by adding a measured amount of paramecia to neutral hydrogen peroxide, and the amount of oxygen liberated in 20 minutes was taken as a measure of the catalase content of the material. A temperature of 5°C. for 24 hours decreased the catalase content of paramecia 38 per cent. Starvation for 8 days decreased it 46 per cent. The paramecia that had been starved for 8 days were fed, and after 24 hours the catalase content had returned to the original normal. The decrease in oxidation in paramecia brought about by low temperatures and starvation is attributed to a decrease in catalase, and the increase oxidation brought about by food to an increase in this enzyme.

*The chemical regulation of the activities of the human kidney.* Edward F. Adolph.

In the experiments reported human subjects ingested certain solutions, and measured the amount and duration of excessive water excretion by the kidneys. It was assumed, as the experiments of others have demonstrated, that the changes in blood composition which followed such ingestions approximated closely the changes which would have occurred if the ingested solutions had been added directly to the blood. These changes in blood composition probably influenced the dis-

criminating tissues of the kidneys by their immediate contact. This study did not include the factors of nervous, vascular, endocrine or drug control.

Ingestion of any dissolved substance in a solution which was hypertonic caused excessive excretion of water, except such substances as sucrose which are changed in metabolism. The kidneys were stimulated by these substances through mobilization of water, since the maximal concentration characteristic of the kidneys limited their excretion without water. None of them are specific diuretics, though they are often stated to be such. Their common property is that of raising the osmotic pressure of body fluids, but it is important to note that only the substance ingested was excreted in this excess of water at a rate much above normal. This type of diuresis was chiefly characterized by the fact that its duration rather than its height varied with the amount of excessive substance.

After drinking water alone a diuresis occurred in which very little except water was excreted at more than the normal rate. This "water diuresis" evidently represents a different type of kidney activity from "salt diuresis." It is characterized by having its height rather than its duration correlated with the amount of water ingested, though there was a limiting rate of about 1 liter per hour. Priestley has shown that the blood is actually diluted, though very slightly, in this type of diuresis.

Further experiments were made in which isotonic solutions of NaCl, KCl, $CaCl_2$, Locke and urea were ingested. Locke and NaCl gave no true diuresis, but a slightly augmented water excretion lasting many hours. This is an edematous retention, characteristic of fluids which simulate in composition the body fluids. KCl and urea, on the other hand, induced very rapid diuresis. In it the height rather than duration was increased with the amount ingested. Evidently the kidneys were affected very differently by balanced and by unbalanced isotonic solutions. In hypertonic solution all substances gave diureses which are of the same type; but in isotonic solutions some gave diureses and others did not. Those which gave diureses added to the blood substances which the kidneys were able to eliminate from the normal concentrations and proportions in the blood. $CaCl_2$ gave no increment in water excretion; which would have to receive a third explanation, that the permeability of the kidney was inhibited.

*On the relations of cortical and sub-cortical cerebral lesions to spastic phenomena in the marsupial.* FRED T. ROGERS.

With the exception of the Monotremes, the Marsupial seems to have the most primitive type of mammalian brain. It has many reptilian resemblances but differs in possessing a true nep-pallial cortex. In this cortex there are locally excitable areas causing movements of the head, neck, facial and fore-leg muscles but none, so far as known, of the hind leg or tail. This cortex is being studied by the method of localized lesions with observation of the immediate and chronic effects.

A lesion by the electro-cautery involving only the excitable areas of the cerebral cortex and not the corpus striatum or thalamus produces the following immediate characteristic effects: there is no marked paralysis of the face or contra-lateral legs, but there is difficulty in executing finely coördinated movements such as running, climbing, walking a narrow board, and handling its food. The contra-lateral legs may be displaced from their normal position and are either not replaced or with difficulty. These effects are temporary and after a few days there is marked recovery of ability to carry out these movements. No constant facial paralysis was observed and no constant alteration of cutaneous reflexes.

A destructve lesion of both the cortex and underlying basal nuclei causes the following additional effects: a tonic distortion of the head to the ipse-lateral side and resulting circus movements when the animal walks. There seems to be complete inability to turn the head in the opposite direction either in walking or to remove irritating objects. There is great difficulty in feeding and the animals become very thin. The fingers of the contra-lateral hand frequently assume a tonic flexed condition. No changes in the grasping reflexes of the tail were observed.

Transection through the anterior part of the mid-brain is followed by typical decerebrate rigidity with extension of the fore and hind legs and elevation of the head.

In the opossum therefore the spastic type of paralysis follows, not from a loss of cerebral cortex but from a sub-cortical injury. Loss of the excitable cortex shows itself only by inability to carry out the finer coördinated movements.

*Heparin, an anticoagulant.* Preliminary Communication. W. H. Howell.

Heparin was first described by Howell and Holt (This Journal, 1918, xlvii, 328). The present note gives a simpler method of preparation which yields a material that is water-soluble, is not injured by boiling, and is without injurious effects when injected intravascularly. One milligram in saline solution prevents the clotting of 5 to 10 cc. of blood (dog, cat) outside the body. Injected intravenously in this proportion it renders the blood incoagulable with no noticeable effect otherwise upon the animal. The effect wears off gradually. Heparin is obtained from the liver. The following directions for its preparation apply only to the liver of the dog. The product obtained by this method contains a variable amount of pigment and other impurities. The active substance may be isolated from the crude preparation by methods which will be described in a forthcoming paper dealing with the properties of heparin. This preliminary communication is intended as an aid to laboratory workers who may need an effective anticoagulant in their experimental work. Those who do not care to make the material may obtain it from Hynson, Wescott and Dunning, Baltimore.

*Method of preparation. 1.* Irrigate the excised liver (dog) through the portal vein with one liter of 1 per cent solution of sodium chloride,

squeezing out the blood also with the hands. Cut into small pieces, rub in mortar to a paste, spread on plates and dry at 150° to 160°F. Powder coarsely and keep in stoppered bottles. If possible this material should be kept several months before being used. *2.* Rub up the dry granulated liver in a mortar with five times its weight of 1 per cent solution of sodium chloride. Add enough ammonia to give a distinct alkaline reaction to litmus paper. Extract for 15 minutes, rubbing occasionally. Strain through double thickness of washed cheese cloth, squeeze with hands, and then centrifugalize the filtrate and filter the supernatant liquid. *3.* Precipitate the filtrate by the addition of an equal volume of acetone, mix well, centrifugalize in stoppered tubes. Decant off the supernatant liquid, drain, gather the residue together. *4.* Boil the residue in excess of methyl alcohol (95 per cent) in a reflux condenser for 2 hours. Throw on filter. Wash once with hot methyl alcohol. Dry residue on the filter paper. *5.* Extract the dry residue with 1 per cent solution of sodium chloride for 15 minutes. Do the extracting by rubbing up in mortar. Centrifugalize. Decant and filter the supernatant liquid. *6.* Precipitate the filtrate by addition of an equal volume of acetone. Mix well. Centrifugalize in stoppered tubes. Decant the supernatant liquid, drain, dry the residue in the tubes. *7.* Dissolve the residue in the tubes in a small volume of distilled water, filter into suitable crystallizing dish or crystal. Evaporate to dryness. The residue should be formed as thin brownish scales, which can be chipped off with a spatula on a sheet of filter paper, powdered in a thoroughly dried mortar and kept in a stoppered, thoroughly dried bottle. If the residue is not scaly but shows some amorphous whitish material, it must be dissolved again in distilled water, filtered from the insoluble residue and again evaporated to dryness. If necessary this process must be repeated until the residue is entirely composed of scales and is entirely soluble in water.

# THE SUPPOSED RELATION OF THE ADRENALS TO REFLEX VOLUME CHANGES IN THE DENERVATED LIMB[1]

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Bayliss (1) showed (in the dog, cat and rabbit) that the volume of a hind limb, denervated by section of the sciatic and anterior crural nerves, undergoes characteristic changes when the central end of a peripheral nerve (the contralateral sciatic) or the peripheral end of the splanchnic is stimulated. During the rise of arterial pressure the volume increases, but this increase is quickly followed by a diminution, which carries the limb volume below its initial value. Similar reactions were obtained with the denervated kidney. Bayliss interpreted the preliminary increase in volume as a passive dilatation due to the increased arterial pressure and the subsequent shrinking as a local reaction "the muscular coat of the arteries reacting, like smooth muscle in other situations, to a stretching force by contraction." He found that the reaction may also be caused when the rise of pressure is induced by asphyxia in a curarized animal, although the effect in this case is possibly complicated by the admixture of the direct action of asphyxial blood on the vessels. When it is caused in this way or by "exciting the central end of a pressor sensory nerve . . . . naturally the vasomotor nerves of the organ must be cut, as also in the case of splanchnic excitation when a viscus supplied by that nerve is under observation." "As regards the limb," he says, "I have not noticed that there is any difference in the reaction whether the nerves are cut or not. The reaction is, therefore, of peripheral origin, and, as I believe, myogenic in nature."

When the blood pressure was caused to fall, as by stimulation of the depressor, there was first a (passive) shrinking of the denervated limb and then a swelling which carried the volume above the initial value.

[1] A summary was presented to the Society for Experimental Biology and Medicine October 19, 1922.

This Bayliss explained as a reaction of the vascular muscle "to a diminution of tension by relaxation." A similar reaction was obtained when a fall of pressure was produced in the hind limb by obstructing the abdominal aorta for a few seconds, and also by obstructing the external iliac artery. In this case too the reaction was not prevented by leaving the connection of the limb with the central nervous system intact. Sometimes a fall of pressure, such as that caused in the limb by temporary clipping of the abdominal aorta, evoked two or more successive reactions, a "reverberation," as he terms it. He completed the proof of the peripheral origin of the reactions by finding that they are obtained in excised arteries. This, he says, "disposes of the objection that the phenomena in question are due to any other cause than the change of tension" and "affords a proof of their myogenic nature."

In discussing the effect of stimulating certain nerves he says that he "noticed that the local reaction is not always present" (2). If we interpret correctly his remark (1) that "in asphyxia one occasionally meets with curves like Fig. 4" (the only one showing the effect of asphyxia), a typical reaction was only sometimes elicited by asphyxia.

The animals, apparently including the cats, were anesthetized with morphine (30 to 130 mgm. subcutaneously) and kept under ACE. In most cases curara was also given.

Anrep (3), (4), using dogs anesthetized with morphine and ACE mixture, repeated the work of Bayliss, and concluded that "the contraction of the blood vessels recorded by Bayliss and regarded by him as a local reaction of the vessel wall to increased internal pressure is due in every case to the action of adrenalin, the secretion of which was increased under the conditions of his experiments." This applies not only to stimulation of the peripheral end of the splanchnic, which is known to cause an increased output of epinephrin, but also to stimulation of the central end of a nerve like the sciatic and to asphyxia. The evidence adduced by Anrep for his conclusion is that the reaction is not obtained after tying off the adrenals or clipping the adrenal veins, nor, in the case of stimulation of afferent nerves or of asphyxia, after section of the splanchnics.

He (4) was unable to confirm the results of Bayliss on the effects of changes of pressure produced mechanically, as by temporary occlusion of arteries, or his results on excised arteries. He convinced himself that "the dilatation of the blood vessels ascribed by Bayliss to the effect of lowering of internal pressures, was due in each case to the direct action on the blood vessel wall of asphyxial products."

Wachholder (5), however, has confirmed the original statement of Bayliss in regard to excised surviving arteries, and has published curves showing that these objects react to an abrupt increase of internal pressure by contraction.

The few observations we have made with momentary occlusion of the abdominal aorta have yielded curves similar to those figured by Bayliss. We have never considered Anrep's "asphyxial products" theory entirely satisfactory. He could have extended the adrenalin hypothesis to include some of the cases considered by Bayliss as examples of local reaction to diminished pressure, e.g., the limb reaction elicited by temporary occlusion of the abdominal aorta, on the assumption that loss of the normal epinephrin content of the blood traversing the limb would suffice to cause a dilatation, a not improbable assumption in view of the fact that the injection of a sufficient amount of adrenalin to double the normal concentration for a few seconds may cause distinct constriction of the denervated limb.

Pearlman and Vincent (6) working with dogs, cats and rabbits (under ether, or morphine and ether, sometimes with curara in addition), found that with stimulation of the peripheral end of the splanchnic the typical constriction of the denervated limb could not be obtained after elimination of the adrenals by clipping the veins or tying off the glands. The same was true of the limb constriction caused by stimulation of the central end of the sciatic nerve. Thus they confirmed Anrep's work on these two points. But in regard to the effects of asphyxia they got different results, and were "unable to obtain tracings showing any significant difference in the behavior of a denervated limb during asphyxia in an animal with adrenals suppressed as compared with one in a normal animal."

Some writers have assumed, with Anrep, that his experiments constitute a proof that electrical stimulation of the central end of the sciatic causes a reflex increase in the rate of epinephrin output from the adrenals. We have already pointed out (7), (8) that this is not the case. All that could be deduced from Anrep's experiments on stimulation of afferent pressor nerves, supposing his results to be correct, is that the adrenals are essentially concerned in the reaction, and that probably the epinephrin given off from them is the important factor.

In repeating the experiments we have been led to the conclusion that even this is too much to assume, since excellent reactions can be obtained after the adrenals have been eliminated. This makes it certain that whether epinephrin is ever a factor in the reaction or not, some other

factor or factors are involved. It is therefore impossible to employ this reaction to determine the question whether an increased output of epinephrin can be reflexly elicited by experimental stimulation of the central end of the sciatic, and all the arguments in favor of such a reflex increase based upon this reaction must be written off. It is easy to show, however, that small quantities of adrenalin, injected at a rate sometimes well within the limits of the ordinary epinephrin output, as determined by us, can elicit good limb reactions. It would therefore be difficult to decide in certain cases whether epinephrin was or was not a factor in the reaction, even where it was perfectly clear that other factors were concerned.

*Technique.* The plethysmograph collar for the hind limb of dogs was made of a section of the inner tube of a small automobile tire, invaginated into the plethysmograph, according to the procedure of Dale and Richards (9), who employed the inner tube of a bicycle tire for cats. We found this quite satisfactory. For cats we generally employed a piece of the tubing used for making rubber bands. In all the animals referred to in the text the vagi were cut, and in some the stellate ganglia were also excised. Blood pressure was recorded in the carotid artery. Drugs and adrenalin solutions (made up in salt solution) were injected into the jugular vein. The left leg was denervated and placed in the plethysmograph, while the central end of the cut right sciatic nerve was placed upon shielded electrodes, which were fastened in the wound and were not disturbed throughout the experiment. Most of our experiments were made on dogs as, in our experience, a greater number of dogs give the reaction (before interference with the adrenals) than is the case with cats. This may depend, partly at any rate, upon the dosage and nature of the anesthetic and the amount of curara given. Even among the dogs there were individuals in which we could not elicit a typical reaction throughout the experiment. If we have correctly interpreted what Bayliss says about this matter, this was also his experience. The dogs were anesthetized with morphine (usually 35 to 50 mgm., in larger dogs, 75 to 100 mgm.) and ether. We never gave morphine to the cats, although apparently Bayliss did so. We do not know whether the excitant effect of this drug in cats might be favorable to eliciting the limb reaction or not. Our cats were anesthetized with ether, except when otherwise mentioned. Both dogs and cats received curara from time to time intravenously. A 1 per cent solution of the same sample of curara was always employed, made up in small quantities at a time, so that it was always fresh. The dose is given in cubic-centimeters of this solution. The aim was to give just enough to prevent movements when the sciatic was stimulated, since we found that the limb response was often suppressed temporarily by larger doses of curara. This is also true for the anesthetics, but this seems so much a matter of course in the case of a reflex response that we should not mention it were it not possible that some of the negative results of other workers, a very short time after removal of the adrenals, may have been due simply to deeper anesthetization during the operation for elimination of the glands. Artificial respiration was started just before or immediately after the curara was given. Figure 1 from

a cat (718) and figure 14 from a dog (729) illustrate the influence of curara on the limb reaction, and figure 2 from the same cat, the influence of the anesthetic. They show the necessity of making numerous, although of course not too frequently repeated stimulations, in order to be certain that a reaction apparently lost after some interference with the adrenals has not merely temporarily disappeared.

About 50 animals were employed in the experiments. In those with lower numbers than 736 a tambour with a rather loose membrane was used for recording the limb volume curve; in 736 and all higher numbers a piston recorder.

Some experiments illustrating our results will now be described.

In cat 718, an old one weighing 4.175 kgm., the anesthetic was ACE. On injection of curara the blood pressure fell while the plethysmograph curve rose, indicating a marked expansion of the denervated limb. This is the practically invariable action of curara even when administered repeatedly in these experiments. The dilatation of the limb is striking because it occurs in the face of a fall of arterial pressure which would tend to cause a passive shrinking. Whether it is due to elimination of a residual peripheral vasomotor tone or to some other cause, we do not venture to speculate. Sometimes there is first a small constriction of the limb as the blood pressure is falling, followed by a dilatation.

Two sciatic stimulations lasting 20 seconds (13 minutes, and 24 minutes after curara) gave no typical limb reaction, i.e., the limb simply dilated as the blood pressure rose, but did not constrict beyond its original volume. This was not due to inability of the limb to react to small doses of adrenalin, for good reactions were caused by the injection of 0.0004 mgm. (always expressed as base) of adrenalin (0.0001 mgm. per kgm. of animal) injected in 10 seconds. This was repeated twice, and one of the curves is reproduced (*8* and *9*, fig. 3). One-fifth of this amount (0.00002 mgm. per kgm.) gave no reaction. The average ordinary output of epinephrin is 0.00025 mgm. per kgm. of cat per minute, and an excellent reaction due to epinephrin would have been obtained had the stimulation of the sciatic increased the rate of output even to twice the normal for as short a time as 10 seconds. The sciatic was again stimulated (29 minutes after the last stimulation, and 14 minutes after the last adrenalin injection). A good reaction was obtained (*16* and *17*, fig. 2). ACE was now given, and the stimulation repeated with a negative result (*21* and *22*, fig. 2). This was repeated with the same result. The anesthetic was now stopped, and 12 minutes later stimulation of the sciatic gave a good reaction (*23* and *24*, fig. 2). More curara was then given, and 3 minutes thereafter the sciatic was stimulated. The limb volume curve showed no trace of the typical

constriction (*27* and *28*, fig. 1). This was not due to inability of the curarized limb to respond to small doses of adrenalin, for injection of 0.0001 mgm. per kgm. in 10 seconds caused a large reaction. Twenty minutes after the curara had been administered, the sciatic was again stimulated, yielding an excellent positive reaction (*32* and *33*, fig. 1); and this was repeated 7 minutes, 23 minutes and 35 minutes later, excellent reactions being obtained each time. The abdomen was now opened, more ACE being given, and the adrenal veins prepared for clipping. The abdomen was then closed. Although no clips had been

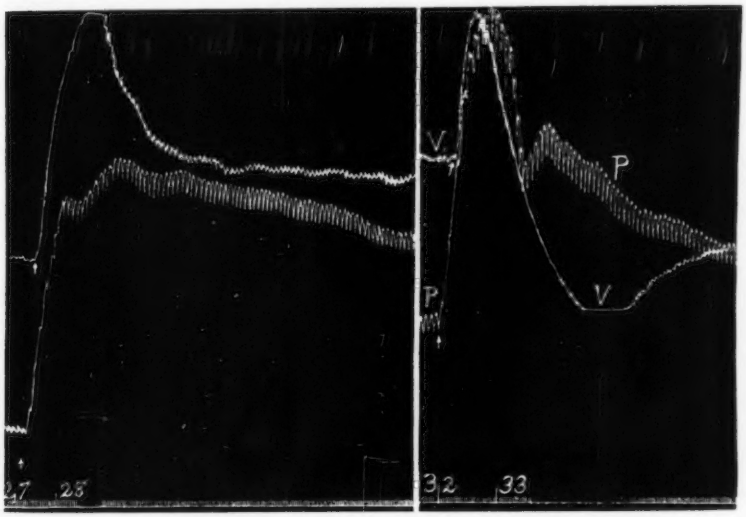


Fig. 1. Tracings from cat 718. Sciatic stimulation; *27-28*, 3 minutes after administration of curara; *32–33*, 20 minutes after administration of curara. Base-line moved up 30 mm. and the figure then reduced to four-fifths. In all the tracings corresponding points on the limb-volume (upper) and blood-pressure (lower) curves are marked by arrows. Where confusion is possible, through intersection of the curves, the blood-pressure curve is indicated by *P* and the limb-volume curve by *V*. Time in seconds. Zero blood-pressure corresponds with base-line of time trace.

placed on the adrenal veins, sciatic stimulation now gave no limb reaction. This, however, gradually returned, and in 17 minutes as good a reaction was elicited as before. More curara was now given, and the reaction disappeared, but 14 minutes thereafter a good reaction was

again obtained. Eight minutes later, stimulation of the sciatic gave the reaction shown in figure 3 (*58* and *59*) with the adrenal veins open. After an interval of 7 minutes, the sciatic was stimulated with the same strength and duration of stimulus but with the adrenal veins clipped (fig. 3, *60* and *61*). The limb reaction was, if anything, stronger than with the veins open. The observations were repeated with the veins open and clipped, with the same result. There was no evidence in this experiment that clipping the adrenal veins affected the reaction. That the clips completely obstructed the blood flow in the veins was always verified by direct observation, both when they were put on and when they were taken off. Further, it was invariably found that a large

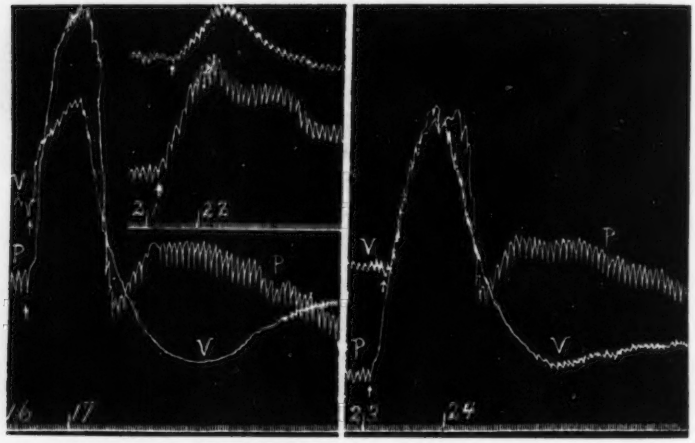


Fig. 2. Tracings from cat 718. Sciatic stimulation; *16–17*, during light anesthesia and curarization; *21–22*, 2 minutes after administration of ACE; *23–24*, 10 minutes after administration of ACE. Base-line moved up 58 mm. in observations *21–22*, and 60 mm. at *16–17* and *23–24*, and the figure then reduced to four-fifths.

epinephrin reaction (constriction of the limb with rise of arterial pressure) occurred when the clips were removed, showing that the epinephrin secreted during the period of clipping had been effectively pent up in the glands. We point this out distinctly because some writers have asserted that epinephrin finds its way freely into the circulation by collateral routes after clipping the adrenal veins. In preparing the veins for clipping we always tie the lumbo-adrenal vein external to the

gland just before it crosses it. When veins are seen which might possibly afford an anastomotic path from the adrenals after application of the clips they are included in the ligature, or tied separately. When the adrenal vein opens into the renal vein, as occasionally happens, the clip is of course placed between the gland and the renal vein.

Figure 4 shows a small sample of the tracings from a dog (724) in which it was also impossible to detect any clear difference in the reaction whether the adrenal veins were clipped or open. The plethysmograph was connected with a tambour, and it must be noted that usually the volume changes of the limb were not so much magnified in this way as when a piston recorder was employed. The animal was a male, weighing 7.75 kgm. Curara was given in two doses of 2.5 cc. each. The first two sciatic stimulations failed to yield a positive limb reaction, although adrenalin in the dose of 0.0035 mgm. (or 0.0005) mgm. per kgm. of

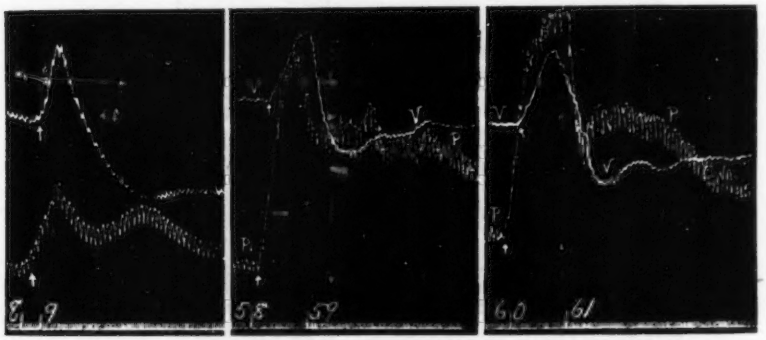

Fig. 3. Tracings from cat 718. Sciatic stimulation; *58–59*, adrenal veins unoccluded; *60–61*, during occlusion of adrenal veins with clips. At *8–9*, intravenous injection of 0.0004 mgm. adrenalin. Base-line moved up 68 mm. in observations *58* to *61* and 41 mm. in observations *8–9*, and the figure then reduced to four-fifths.

body weight) in 10 seconds gave a good reaction, and one-tenth of this amount a small but definite reaction. About 15 minutes after the last dose of curara sciatic stimulation began to yield positive limb reactions, which became more decided in 20 minutes more, and could always be elicited thereafter during the 3 hours for which the experiment was still carried on. The reaction was not affected by clipping the adrenal veins, as illustrated in figure 4 (*41* and *42*, *43* and *44*). The same was true after excision of the right adrenal; the reaction was as

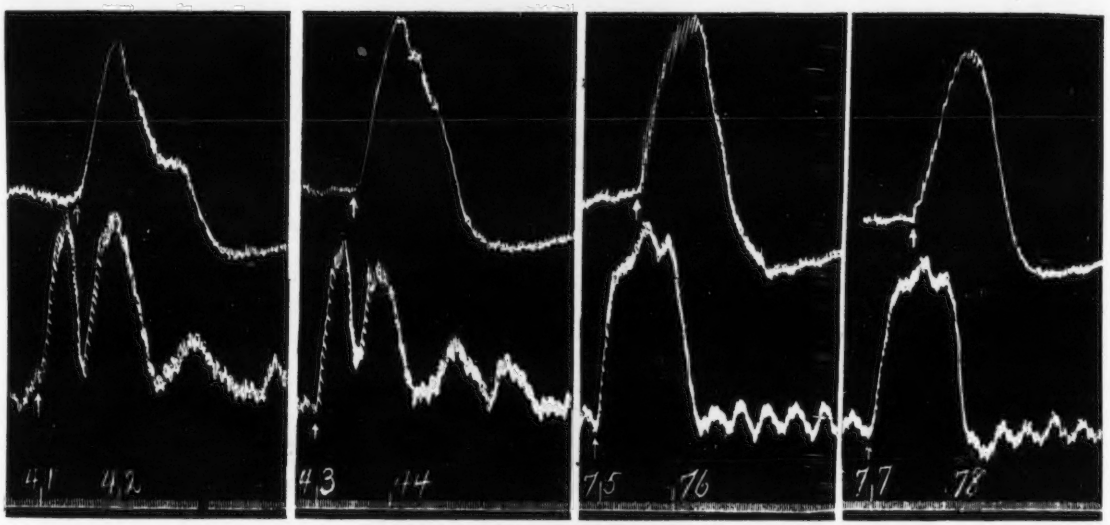


Fig. 4. Tracings from dog 724. Sciatic stimulation; *41–42*, adrenal veins unoccluded; *43–44*, during occlusion of adrenal veins with clips; *75–76*, after excision of right adrenal; *77–78*, during occlusion of vein of remaining adrenal. Base-line moved up 45 mm. in observations *41* to *44* and 32 mm. in observations *75* to *78*.

large as when both adrenals were present, and clipping of the remaining adrenal vein produced no effect upon the result of sciatic stimulation. At this stage even as small a quantity of adrenalin as 0.0002 mgm. (0.000025 mgm. per kgm. of body weight) injected in 10 seconds gave a definite constriction of the limb. About 3 hours after the first sciatic stimulation, a series of stimulations during which the remaining (left) adrenal vein was alternately open and clipped gave the same results, as illustrated in figure 4 (*75* to *78*). A good reaction was yielded when the sciatic was stimulated after the left adrenal vein had been clipped off for 10 minutes. The left adrenal was now excised. Sciatic stimulation still gave a good constriction of the limb, as also stimulation of the left splanchnic in the thorax.

In figures 5 and 6 are reproduced a specimen of the tracings from dog 737, as an illustration of those taken with the piston recorder. The animal was a male, weighing 7.55 kgm. Curara was given cautiously in doses of 1.5 cc., three doses in all being required in an experiment lasting 4 hours from the time tracings began to be taken. The first two sciatic stimulations did not yield a positive limb reaction. Thereafter an excellent reaction was repeatedly obtained, and no essential difference was produced by clipping the adrenal veins (fig. 5, and two similar pairs of observations taken before *31*, not reproduced). When the clips were removed a large reaction was obtained from the pent up epinephrin, the limb constricting markedly after a slight dilatation synchronous with the small rise of blood pressure. The adrenal veins were now tied, and 2 minutes thereafter the sciatic was stimulated with the result seen in figure 6 (*37* and *38*). Then both adrenals were excised. It should be noted that although the adrenal veins were tied between the gland and the cava, the lumbo-adrenal veins having been previously tied at the outer edge of the adrenals, the removal of the glands, which could not have been discharging any appreciable amount of epinephrin, or the manipulations incident to their removal caused a fall in the blood pressure from 128 mm. to 67 mm. of mercury. The fall could not be due to the anesthetic since no ether had been given for some time. This shows how risky it is to attribute to the loss of epinephrin any change which occurs after removal of the adrenals (fall of blood pressure, diminution in heart rate, etc.) as is the habit with some writers. When the adrenals are excised early in an experiment a fall of pressure is not so commonly observed, nor when present is it usually as great as when the adrenalectomy is done after the animal has been worked with for a while.

Two minutes after excision of the adrenals the sciatic was again stimulated with the result shown in figure 6 (*39* and *40*). Thirteen minutes thereafter the sciatic was again excited and an even larger reaction was obtained (not reproduced). Whatever the mechanism of these reactions may be, it is obvious that the adrenals are not essential since they can be obtained in great intensity in the absence of these glands. A large reaction was now elicited by the injection in 10 to 12 seconds of 0.0004 mgm. (0.00005 mgm. per kgm. of body weight) of adrenalin. This is equal to the average normal rate of output. Eighteen minutes later, the injection in 12 seconds of only 0.00016 mgm. (0.00002 mgm. per kgm) of adrenalin

gave a good limb reaction. This is only half the normal average rate of output of epinephrin. It is clear that a very slight and brief increase in the epinephrin concentration of the blood coming to the limb is capable of yielding a reaction. Yet in this experiment the reaction which was obtained by stimulation of the sciatic was so little influenced by the epinephrin factor (while the adrenals were still intact) that it was not possible to demonstrate any definite difference after removal of the glands. This is in flat contradiction to the view that excitation of the central end of the sciatic causes an outburst of epinephrin.

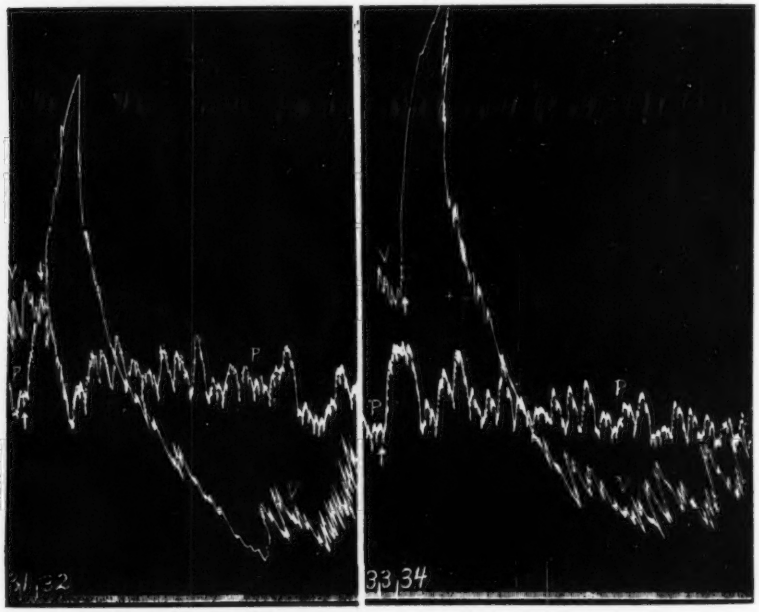


Fig. 5. Tracings from dog 737. Sciatic stimulation; *31–32*, adrenal veins unoccluded; *33–34*, during occlusion of adrenal veins with clips. Base-line moved up 35 mm. and the figure then reduced to four-fifths.

Since Anrep in many of his experiments removed the stellate ganglia, in addition to dividing the vagi, it was considered advisable to determine whether the results were altered in any way by this procedure, although, apart from the additional trauma, it is difficult to see how removal of the stellates could exercise any influence upon the reaction we were studying. Vincent contented himself with dividing the vagi, and it does not appear that Bayliss did even that as a matter of routine.

We failed to observe that removal of the stellates caused any difference in the ease with which a good limb reaction could be evoked after elimination of the epinephrin output of the adrenals. Figure 7 gives a specimen of tracings obtained from a female dog (761), weighing 8.76 kgm. The vagi were cut and the stellate ganglia excised. Curara was given in two doses (3.5 cc. in all). The first two sciatic stimulations, a few minutes after the second dose of curara, did not yield a positive limb reaction, i.e., there was no constriction beyond the initial

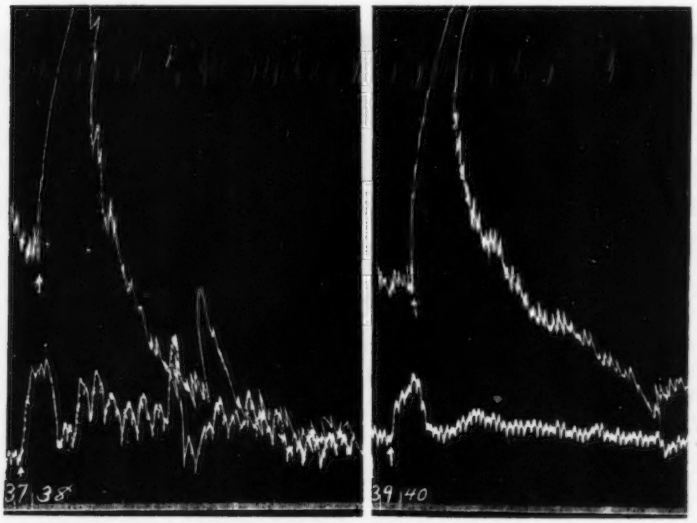


Fig. 6. Tracings from dog 737. Sciatic stimulation; *37–38*, after tying off both adrenal veins; *39–40*, after excision of both adrenals. Base-line moved up 53 mm. at *37–38* and 20 mm. at *39–40*, and the figure then reduced to four-fifths.

volume succeeding the passive dilatation. At this time it also required a relatively large dose of adrenalin to give a reaction, 0.0013 mgm. (0.00015 mgm. per kgm.) injected in 10 seconds causing only a small constriction, while 0.0033 mgm. (0.0004 mgm. per kgm.) injected in 15 seconds gave a large reaction. Twenty-three minutes after the second dose of curara, stimulation of the sciatic began to yield good reactions. The right adrenal was now excised, the left adrenal vein prepared for clipping, and the sciatic stimulated (*16* and *17*, fig. 7)

with the vein open. Eight minutes later the stimulation was repeated with the vein clipped (*19* and *20*, fig. 7). The clip was placed on the vein about one minute before stimulation was begun. It will be seen that neither the limb reaction nor the rise of blood pressure was affected by clipping the adrenal vein. When the clip was taken off a good reaction was caused by the pent-up epinephrin. The observations were repeated, the clip being applied 2 minutes before sciatic stimulation. There was no essential difference between the curves whether the vein was open or clipped. When the clip had been left on for 4

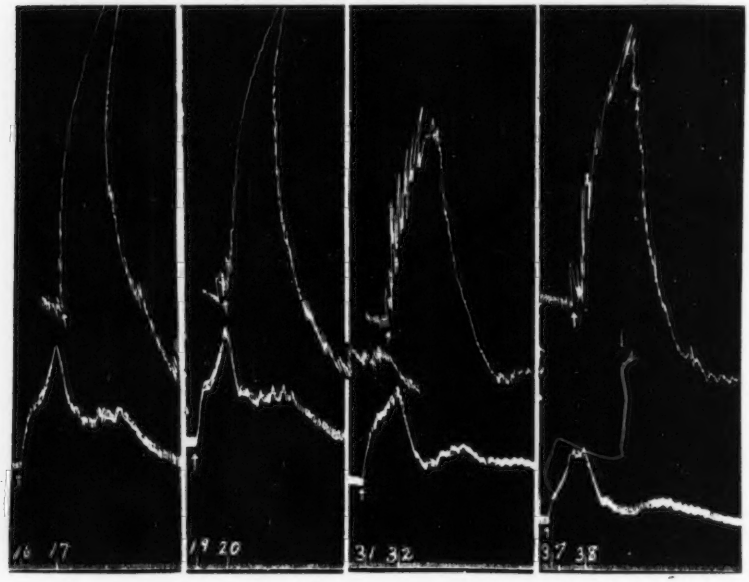


Fig. 7. Tracings from dog 761. Sciatic stimulation; *16–17*, after excision of right adrenal; *19–20*, during occlusion of the vein of the remaining adrenal with a clip; *31–32*, after tying off vein of remaining adrenal; *37–38*, after excision of remaining adrenal. Base-line moved up 18 mm., and the figure then reduced to two-thirds.

minutes, stimulation of the sciatic still gave approximately the same reaction. Two minutes after removing the clip sciatic stimulation gave a reaction quite similar to that obtained before. The left adrenal vein was then tied off, and stimulation of the sciatic repeated. A good

positive limb reaction was elicited (*31* and *32*, fig. 7), quite as good as that yielded 9 minutes before with the adrenal vein open. The left adrenal was now excised, and 9 minutes thereafter the sciatic was stimulated (*37* and *38*, fig. 7). A good reaction was obtained, although both adrenals had been removed. More curara (2 cc.) was now injected and the reaction could not thereafter be elicited for some time. Later on, stimulation of the sciatic gave typical limb reactions (constriction following passive dilatation).

To avoid the necessity in the actual experiment of interfering with the adrenals, a number of animals were subjected to operations calculated to suppress permanently the epinephrin output of the glands, and allowed to recover. If the reactions studied by Bayliss and Anrep have been correctly interpreted by the last named observer as due entirely to a reflexly increased epinephrin output from the adrenals, they ought not to be obtained in such animals. We find that they can be well obtained. Therefore we cannot accept the supposed proof of a reflexly increased epinephrin secretion caused by electrical stimulation of the central end of the sciatic. Figure 8 shows specimens of the tracings from dog 766, a male, weighing 8.1 kgm. The right adrenal was removed and, 12 days later, about three-quarters of the left, the central portion on each side of the lumbo-adrenal vein being spared. The fragment was denervated by cutting the nerves between the coeliac ganglion and the gland without dividing the splanchnic, so as to leave the conditions as favorable as possible for a good reflex rise of blood pressure in the plethysmograph experiment. The experiment was performed 38 days after the second operation. The dog then weighed only 6.7 kgm. but appeared to be in good health. Sciatic stimulation did not at first yield a positive limb reaction, but began to give good reactions as the curara paralysis became less deep. This is what is often observed in animals in which there has been no interference with the adrenals, and indicates that it is not paralysis of the efferent epinephrin-secretory path by curara (10) which is responsible for the failure to obtain the reaction at this stage. Adrenalin gave a good reaction in a dose of 0.0006 mgm. (0.0001 mgm. per kgm.) injected during 12 seconds, and an enormous reaction in a dose of 2½ times that amount injected during 10 seconds. At this stage stimulation of the sciatic with a weak stimulus (8 cm. distance between the coils) gave a positive although not a strong reaction (*26* and *27*, fig. 8), whereas a stronger stimulus (6 cm.) for the same length of time (10 seconds) gave a much larger reaction (*28* and *29*, fig. 8). It scarcely needs to be mentioned

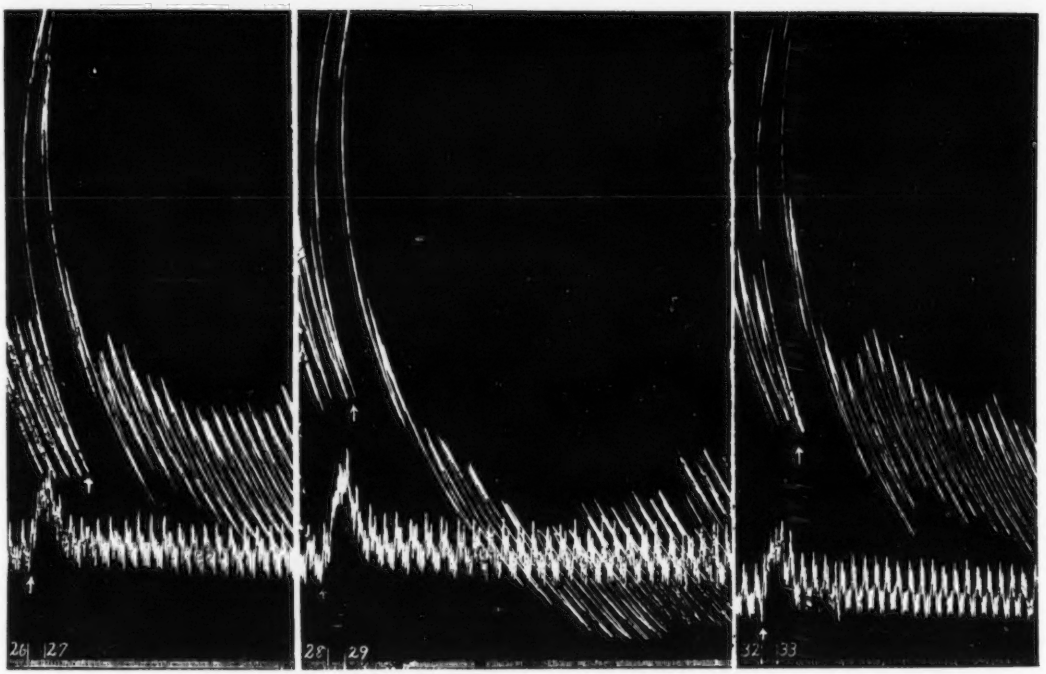


Fig. 8. Tracings from dog 766. Right adrenal excised, three-quarters of left adrenal excised and remaining fragment denervated, 5½ weeks previously; sciatic stimulation, *26–27*; *28–29*, stronger stimulus; *32–33*, after excision of remaining (denervated) fragment of adrenal. Base-line moved up 11 mm. and the figure then reduced to three-fourths.

that a stronger stimulus does not always elicit a larger reaction of the kind studied in this paper than a weaker stimulus. That entirely depends upon circumstances, and the same is true of the duration of the stimulus. It is therefore necessary to vary the strength and duration of the stimulus to see whether a reaction can be evoked. In some animals almost any duration and strength of stimulus will suffice to elicit a good reaction, and then the stimulus can be kept constant throughout the experiment. After removal of the fragment, which weighed 0.25 gram and had probably undergone little hypertrophy, a fair limb reaction was obtained (*32* and *33*, fig. 8). More curara was now given and the reaction could not be elicited for a time. It then returned, and excellent reactions were yielded with sciatic stimulation, whenever applied during the remaining two hours of the experiment.

As another example of the experiments in which the animals were allowed to recover from operations for suppression of the epinephrin output, dog 764 may be referred to. The animal was a female, weighing 10.15 kgm. at the time of the first operation (excision of the right adrenal) and 9.07 kgm. 16 days later, at the time of the second operation (excision of two-fifths of the left adrenal, with curetting out of the remaining medulla and denervation of the remnant). The experiment was made 34 days after the second operation, and in the same way as in dog 766, except that the right splanchnic was also stimulated in a number of observations toward the end of the experiment. The dog was in good condition and had regained its original weight (10.2 kgm). The rise of pressure produced by sciatic stimulation was rather small throughout the experiment. We are unable to say whether this was the reason for the negative results at first obtained as regards the limb reaction. But later on good reactions were elicited by each sciatic stimulation. An instance is given in figure 9 (*66* and *67*, confirmed by at least 10 similar observations not reproduced). Stimulation of the right splanchnic in the thorax also gave good reactions (*80* and *81*, fig. 9, and other observations not reproduced). The remnant of the left adrenal was now excised. It had undergone little, if any, hypertrophy. It weighed 0.56 gm., about three-fifths of the original weight of the entire left gland. The right splanchnic was again stimulated. The first two excitations failed to yield a positive limb reaction, but succeeding stimulations gave good positive reactions (*88* and *89*, fig. 9, and other observations not reproduced). The injection of 0.0035 mgm. of adrenalin (0.0003 mgm. per kgm.) in 12 seconds gave a very large reaction, far larger than any of the reactions obtained by sciatic or splanchnic stimulation. This was also true of the injection of 0.0015 mgm. of adrenalin in 10 seconds.

In other experiments it was also observed that elimination of the adrenals did not prevent definite limb reactions from being obtained on stimulation of the splanchnic. An example is shown in figure 10 (*88* and *89*). In this experiment on dog 754, a female, weighing 6.15 kgm., stimulation of the sciatic having been shown to elicit positive limb

reactions, the right adrenal was excised and a series of observations made in which the left adrenal vein was alternately clipped and open. In both cases positive reactions were obtained. Sometimes it seemed that these were less marked when the left adrenal vein was clipped during the stimulation than when it was open. But more than once a second stimulation with the adrenal vein still clipped elicited a better reaction than the first. Removal of the clip caused always a large limb reaction, and there was practically no difference in its magnitude when the sciatic had been twice stimulated during a period of 7 minutes' occlusion (fig. 11, observation *37*) and when the vein was released after a period

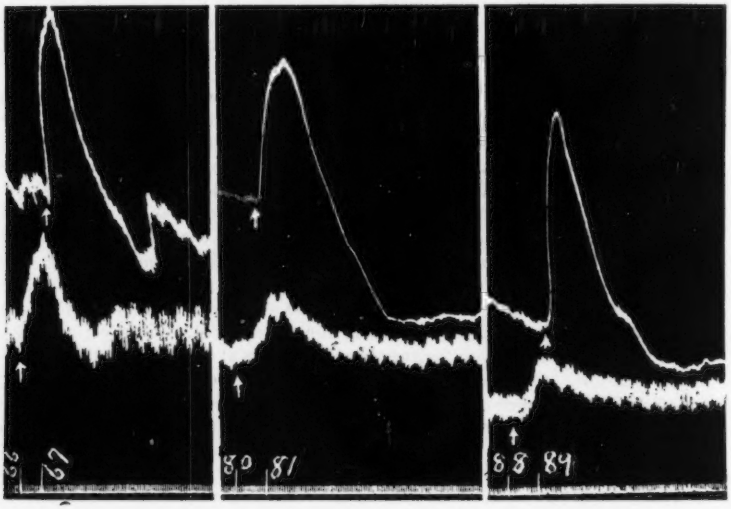


Fig. 9. Tracings from dog 764. Right adrenal and two-fifths of left adrenal excised, medulla of remaining adrenal fragment curetted out and the fragment denervated five weeks previously; *66–67*, sciatic stimulation; *80–81* (right) splanchnic) stimulation; *88–89*, splanchnic stimulation, after excision of remaining (denervated) fragment of adrenal. Base-line moved up 15 mm.

of 6 minutes' occlusion without stimulation of the sciatic, except that probably the limb volume curve began to rise again somewhat sooner in the latter case, corresponding to the somewhat shorter period during which the spontaneous secretion of epinephrin had been going on (fig. 11, observation *38*). The pent-up epinephrin spontaneously secreted by the gland was accordingly not sensibly increased by stimulation of

the nerve. Other sets of similar observations gave the same result. Twenty minutes after observation *38* (fig. 11) the remaining (left) adrenal was excised, and more curara was given. Stimulation of the sciatic ceased to yield positive limb reactions, but after a time they returned, as shown by such observations as *65* and *66* in figure 10, made 50 minutes after removal of the second adrenal.

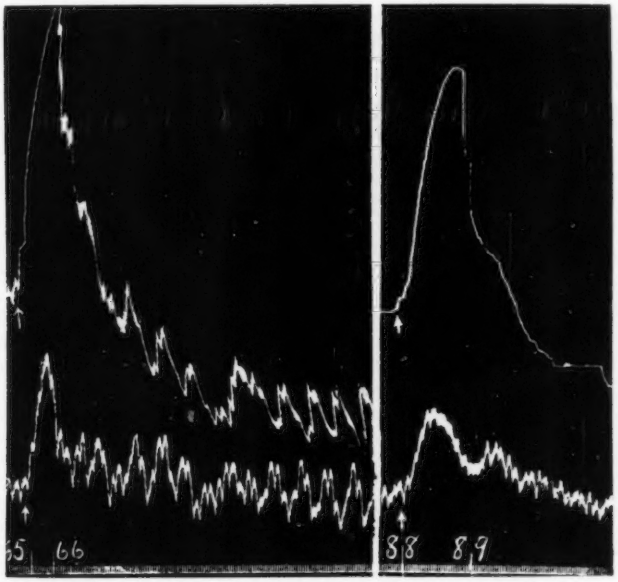


Fig. 10. Tracings from dog 754. *65–66*, sciatic stimulation, after excision of both adrenals; *88–89*, splanchnic stimulation, after excision of adrenals. Base-line moved up 38 mm. at *65–66* and 18 mm. at *88–89*.

It will be noted that in figure 11 the release of the pent-up epinephrin, although it caused such an apparently large constriction of the denervated limb, produced little if any change in the blood pressure. This is an illustration of the fact already pointed out that the vasoconstriction is greatly magnified on the volume curve as traced by the piston recorder. This is probably one reason why some writers who have not compared the reactions obtained by the injection of adrenalin in definite amounts, have yielded to the idea that such large reactions must be due to considerable outbursts of epinephrin. Yet the big volume changes shown in figure 11 were produced by epinephrin secreted by a single adrenal at the ordinary rate and uninfluenced by electrical stimulation of afferent nerves.

Indeed it may be assumed that in the time of occlusion the blood pent up in the adrenal probably could not, owing to the rapid increase in epinephrin concentration, have been charged with nearly as much epinephrin as would have been secreted in the same period had the blood been able to escape. For this reason and also because of the magnitude of the reactions it may be objected that an increased output of epinephrin due to stimulation of the nerve might be difficult to detect in this way. Nor do we imagine that anything like an exact quantitative comparison can be thus made. In previous experiments (11) we have, in making similar comparisons, utilized the relatively considerable space of a long cava pocket for collecting the adrenal vein blood. All we desired to show by the experiment under discussion was that sciatic stimulation did not, at any rate, grossly affect the reaction produced by the naturally secreted epinephrin, as liberated in this way. That the blood pressure did not rise appreciably when the pent up adrenal blood was released presents no difficulty, since the injection of as much as 0.0008 mgm. of epinephrin in 15 seconds (i.e., at the rate of more than twice the average normal output for both glands) likewise caused no appreciable rise of pressure, although a large constriction of the limb.

It is of interest to reproduce, for the sake of the contrast, a few tracings from dog 758, a female, weighing 5.7 kgm. The stellate ganglia were excised in addition to section of the vagi. Stimulation of the sciatic was first shown to yield definite positive limb reactions. A stimulation of the nerve for 30 seconds with a rather weak stimulus gave a limb reaction of about the same magnitude as the reaction produced by injection of 0.0035 mgm. (0.0006 mgm. per kgm.) of adrenalin in 20 seconds, larger than that produced by the injection of 0.0014 mgm. adrenalin in 10 seconds, and much smaller than that caused by injection of 0.007 mgm. in 12 seconds. The limb was therefore relatively insensitive to adrenalin. The right adrenal was then excised and the left adrenal prepared for clipping. The blood pressure fell very little during the operation. The left adrenal vein was now clipped for 2 minutes, and a gradual fall of blood pressure ensued, which lasted till the clip was removed. The total fall of pressure was 22 mm. of mercury. On removal of the clip the pressure rose 40 mm., and the heart rate increased 20 beats a minute. This observation was repeated several times, the central end of the sciatic being stimulated alternately with the adrenal vein open and clipped. Positive limb reactions were obtained in each case. Always on removal of the clip a good rise of pressure occurred, accompanied by a positive limb reaction. No definite difference in the reactions elicited by releasing the adrenal vein when the sciatic had not been stimulated and when it had been stimulated during occlusions of the same duration could be made out (fig. 12). During the occlusion the pressure fell slowly, for instance, in the 3 minutes' occlusion before *31* (fig. 12) the fall of pressure was 26 mm. of mercury. This is emphasized, because as we have previously pointed out (12), it seems easier to demonstrate an influence of withdrawal or restoration of the ordinary output of epinephrin when the cardioregulative nerves have been eliminated than when they are intact. While in figure 12 the rise of pressure at *31* is less steep and the maximum reached somewhat less than at *29*, this difference was not seen in all the observations, and has probably nothing to do with the fact that stimulation of the sciatic (for 30 seconds) occurred during the occlusion before *29*. The limb reaction is somewhat more pronounced at *31* than at *29*. Adrenalin now gave larger reactions (both limb

and blood pressure) than earlier in the experiment. For example, 0.0014 mgm. (0.0002 mgm. per kgm.), injected in 15 seconds, gave a distinct effect.

The left splanchnic was now prepared for stimulation in the thorax. It gave no positive limb reactions with the adrenal vein either open or clipped. But when the nerve was stimulated for 30 seconds with the adrenal vein clipped a big reaction occurred on release of the vein. The occlusion had lasted for 3 minutes (fig. 13, observation *49*). The pressure rose 50 mm. of mercury, and the lever of the piston recorder descended till it rested on the top of the cylinder. The limb reaction was greater, but the maximum rise of pressure was no greater than that caused by injection of 0.0035 mgm. of adrenalin in 10 seconds (*59* and *60*, fig. 13). The left adrenal had been excised and the left splanchnic cut in the

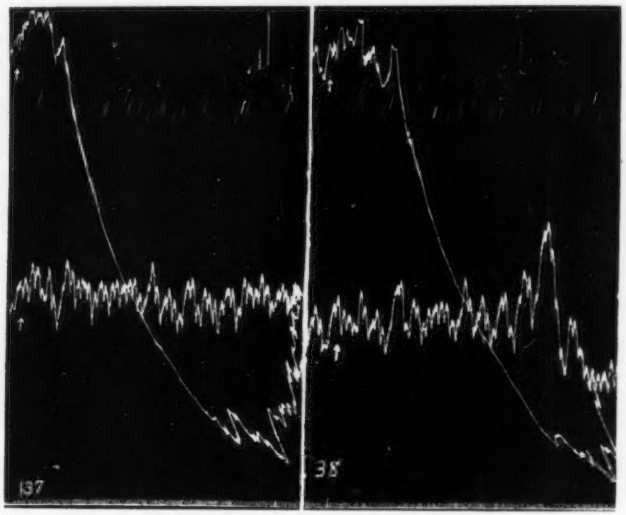


Fig. 11. Tracings from dog 754. At *37* the clip was removed from the vein of the remaining (left) adrenal (right had previously been excised), after 7 minutes' occlusion, during which the sciatic was stimulated for one period of 10 seconds and another (stronger stimulus) of 15 seconds; at *38*, the left adrenal vein was released after 6 minutes' occlusion during which there was no stimulation of sciatic. Baseline moved up 18 mm., and the figure then reduced to three-fourths.

thorax before injection of the adrenalin. Stimulation of the peripheral end of this nerve (the experiment having lasted 4½ hours) now gave practically no rise of blood pressure and no limb reaction, although several trials were made.

From the calibration of the piston recorder (fig. 13) the maximum reduction of volume of the part of the limb in the plethysmograph, whose volume was 215 cc.,

under the influence of the dose of adrenalin injected at *59* to *60* was no more than about 0.25 cc. If the blood in the limb amounted to 5 per cent of its volume this would give a reduction of little more than 2.5 per cent in the blood. In the reaction caused by releasing the pent-up epinephrin (*49*, fig. 13) the reduction would be several times greater. But even this represents only a very moderate vasoconstriction, and it is the great magnification of the volume change obtained with the piston recorder which makes the reactions look so impressive. The excursions corresponding to a change of volume of 0.5 cc. at different points of the arc described by the writing point are, of course, reduced with the rest of figure 13.

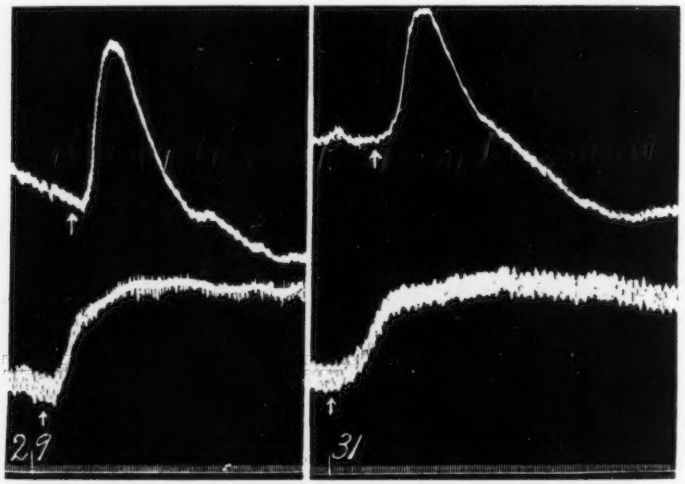


Fig. 12. Tracings from dog 758. At *29*, removed clip from left adrenal vein (the right adrenal had been previously excised), after 3 minutes' occlusion, with sciatic stimulation for 30 seconds during the occlusion; at *31*, removed clip from left adrenal vein after 3 minutes' occlusion without sciatic stimulation. Base-line moved up 30 mm.

In one of the dogs (729) we saw results similar to those of Anrep. That is to say, a positive limb reaction on sciatic stimulation could not be obtained throughout the experiment when the vein of the left adrenal (the right gland having been previously excised) was occluded (figs. 14 and 15). On repeating the stimulation with the vein open the reaction was again elicited.

The animal, a male, weighed 6.55 kgm. The plethysmograph was connected with a tambour. Curara (2 cc.) was given and the sciatic stimulated from time to time, but for the next 50 minutes no positive

limb reaction was obtained (for example, observations *19* and *20*, fig. 14). Adrenalin gave a large reaction (fig. 14, observations *17* and *18*) when injected in the amount of 0.0018 mgm. (or less than 0.0003 mgm. per kgm. of body weight) in 8 seconds. As the curara action was wearing off fair limb reactions began to be elicited by sciatic stimulation (for example, *30* and *31*, fig. 14). More curara (1.5 cc.) was administered, and the reaction, as usual, was much diminished or abolished (*34* and *35*, fig. 14). The right adrenal was now excised and the left adrenal vein prepared for clipping. With the vein open stimulation of the sciatic caused a fair reaction (*36* and *37*, fig. 15) not inferior

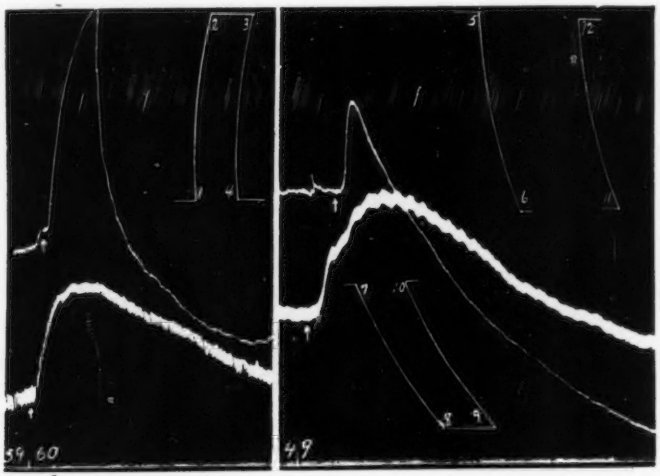


Fig. 13. Tracings from dog 758. At *59–60*, intravenous injection of 0.0035 mgm. adrenalin; *49*, removed clip from left adrenal vein (the right adrenal had been previously excised), after 3 minutes' occlusion with stimulation of the left splanchnic, for 30 seconds during the occlusion.

Beginning at about the same level as the beginning of the volume curves, 0.5 cc. of water was injected into the plethysmograph at *1* to *2* and withdrawn at *3* to *4*. From this point the curve was continued downward by withdrawal of another 0.5 cc. at *5* to *6*, and still continued downward by withdrawal of another 0.5 cc. at *7* to *8*. The process was then reversed, 0.5 cc. of water being reinjected at *9* to *10*, and the curve continued upwards at *11* to *12* by another injection of 0.5 cc. To save space, the portions of the curve *5* to *6* and *11* to *12* have been removed from their original positions and the portion *7* to *8* and *9* to *10* displaced slightly upward. The drum was stationary while recording each trace. The base-line was moved up 18 mm. and the entire figure then reduced to two-thirds.

to that obtained before the excision of the right adrenal (compare, for instance, *30* and *31*, fig. 14). Although we do not think that such reactions can be quantitatively compared by inspection, except roughly,[2] we have frequently observed that removal of one adrenal, which ought, if the reaction is due to reflexly increased epinephrin output, to diminish it approximately to half, has no noticeable influence upon its magnitude when any transient disturbing influence of the operative procedure, if such be present, has been allowed to pass off. When the stimulation of the sciatic was repeated with the left adrenal vein clipped no positive limb reaction was obtained (*38* and *39*, fig. 15). On repeating the

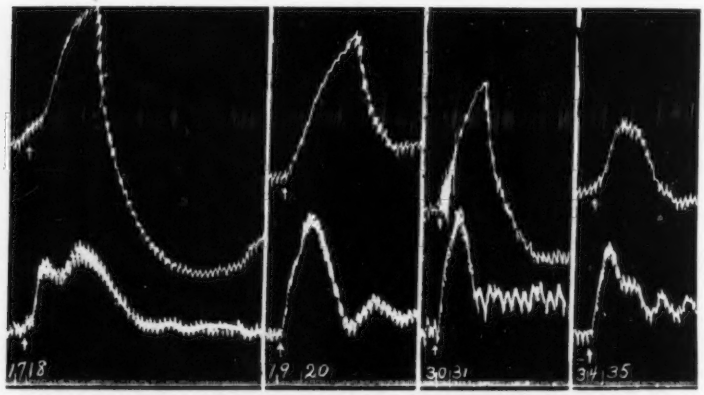


Fig. 14. Tracings from dog 729. Sciatic stimulation; *19–20*, during effective curarization; *30–31*, during disappearance of curara effect; *34–35*, 3 minutes after another injection of curara. At *17–18*, intravenous injection of 0.0018 mgm. adrenalin. Base-line moved up 50 mm. and the figure then reduced to two-thirds.

stimulation with the adrenal vein open, only a small limb reaction was got (*40* and *41*, fig. 15), decidedly smaller than that yielded with the vein open in observations *36* and *37*. However, with a longer time of stimulation (30 seconds instead of 10 seconds) a good limb reaction was elicited with the adrenal vein open (*42* and *43*, fig. 15). The reaction was much better than was given with both adrenals discharging at an earlier period of the experiment. The vein was now clipped and

[2] When adrenalin is injected in definite amounts and at a definite rate there is a fairly regular graduation of the magnitude of the reaction according to the amount injected.

stimulation repeated for the same length of time, but the limb reaction was negative (*44* and *45*, fig. 15, confirmed by another observation not reproduced). Another stimulation was now made with the adrenal vein open, and a fair positive reaction elicited. The limb reaction was less than that given by injection of 0.0014 mgm. (0.0002 mgm. per kgm.) of adrenalin injected in 10 seconds. The next stimulation of the sciatic with the adrenal vein open yielded a reaction which was so slight as to be doubtful, and stimulation with the vein clipped was negative. More curara (1 cc.) was given, and negative reactions were obtained with the vein open. But after 7 minutes a fair positive reaction was again

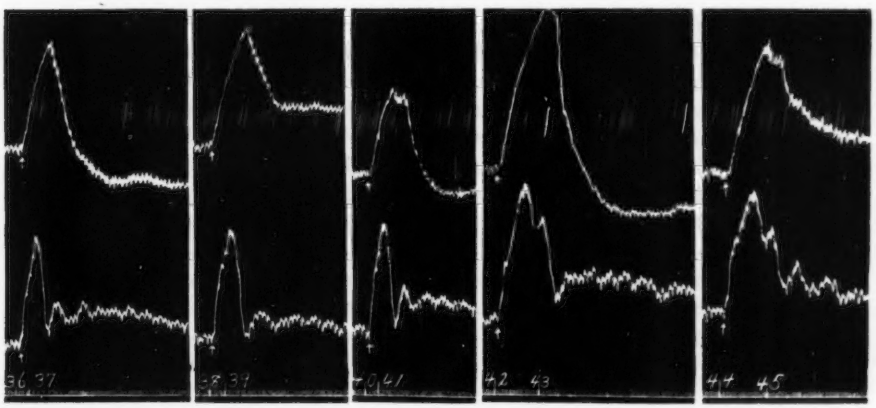


Fig. 15. Tracings from dog 729. Sciatic stimulation; *36–37*, after excision of right adrenal; *38–39* and *44–45*, with vein of remaining adrenal clipped; *40–41*, remaining adrenal vein unoccluded; *42–43*, adrenal vein unoccluded but stronger stimulus. Base-line moved up 40 mm. and the figure then reduced to two-thirds.

elicited when the vein was open, although none with the vein clipped. Removal of the clip always caused a good reaction. During the remainder of the experiment (half an hour) the limb reactions were negative during sciatic stimulation with the adrenal vein open.

This is a type of experiment which has been rare in our experience in dogs, and therefore it has been described rather minutely. If it were the common type we should conclude that something coming off in the adrenal blood, almost certainly epinephrin, was essentially concerned in the limb reaction. For reasons already mentioned, and the point will be again alluded to, we do not think the experiment would in

any case prove that the rate of epinephrin output was increased by stimulation of the central end of the sciatic. Since, however, artificially injected adrenalin in such quantities as might normally be coming off from the glands and also epinephrin released in the adrenal vein blood after a period of occlusion yield excellent reactions of a similar nature, there is no reason to deny that sometimes epinephrin might be a factor in the reaction. If this is so, it is natural to assume that under different circumstances the importance of this factor may vary in relation to other factors. That other and more constantly acting and more important factors are involved is clearly shown, we think, not only by the experiments of Bayliss, some of which are unintelligible on the adrenalin hypothesis, but by the fact that in the great majority of our own animals it was possible to elicit, and often many times, good reactions, when the adrenals had been eliminated by excision or by occlusion of the veins. Frequently these reactions were as large as, sometimes indeed even larger than when the adrenals were normally connected with the circulation. As to the nature of these other factors, we shall only remark that the one emphasized by Bayliss, the local reaction of the vessels to changes of pressure, can no longer be waved aside on the ground of such experiments as those of Anrep.

It seems in any case obvious that a reaction which in the great majority of dogs, under the conditions of our experiments, is so little related to the epinephrin output of the adrenals that it can be as well obtained when they have been eliminated as when they are present, cannot be used as evidence that experimental stimulation of the central end of the sciatic causes an increase in the rate of output of epinephrin. The reaction is so readily evoked by the injection of small doses of epinephrin, lying sometimes within the range of, or not greatly exceeding the amounts given off normally, that any considerable increase in the output could not fail to produce so great an effect that elimination of the adrenals must inevitably be associated with a marked and permanent diminution in the reaction, or its total suppression.

As already pointed out, even if it had been true that the typical limb reaction was never obtained on excitation of the sciatic in the absence of the adrenals, this would not of itself prove that stimulation of the nerve increased the output of epinephrin. The presence of some epinephrin in the circulating blood might be necessary to facilitate the reaction without any change occurring in its concentration on stimulation of the nerve. Or the average concentration of the epinephrin in the blood passing through the limb might be raised in at least two ways without any change having occurred in the output of epinephrin per unit of time: First, by an actual change in the concentration of epinephrin in the blood

of the anterior segment of the inferior cava where the adrenal blood mingles with that from the splanchnic area, associated with vasomotor changes in this area. Such a change in concentration might be reinforced by the increased blood flow through the adrenals causing a more rapid sweeping out of the blood already in the glands, charged with the higher content of epinephrin corresponding to the slower blood flow at the moment of stimulation. A change of concentration need only last for a very brief time in order to evoke a reaction from an object so sensitive as the denervated limb. Secondly, the increased flow through the denervated limb, under the influence of the higher arterial pressure, would offer to the vessels of the limb a larger supply of epinephrin per unit of time, and therefore since that substance is rapidly destroyed in passing through the tissues, a higher average concentration throughout the vascular path.

Let it be repeated, our results show that such factors could rarely be prominent, much less decisive factors in the reaction, else it would be impossible that elimination of the adrenals should not greatly alter it. They need only be brought into the reckoning in those cases where interference with the adrenals does seem markedly to alter the result of sciatic stimulation. And before anyone can conclude from such reactions that excitation of these afferent fibers really causes an increased secretion of epinephrin, they must not only be brought into the reckoning, but eliminated from it by proper control experiments, if such can be devised.

In connection, however, with results such as those obtained on dog 729, the question might be raised whether in applying clips to the adrenal veins some mechanical interference with the flow in the cava, and therefore with the outflow from the limb might not have occurred. Such interferences might conceivably render the limb constriction less easy. There is no evidence of any such mechanical factor in our experiments. Any sensible interference with the cava flow would, it is to be assumed, be at once reflected in distortion of the volume curve. But this was not observed. A more important question is whether under other conditions as to anesthesia, curarization, blood pressure level, etc., or if the experiment had been carried on for a longer time, positive limb reactions might not have been obtained here also after elimination of the adrenals. This question cannot of course be definitely answered. But it may be pointed out again that not infrequently a reaction which may seem to have been reduced or abolished by elimination of the adrenals is well obtained later on in the experiment. This is further illustrated in figures 16 and 17 from dog 748.

The animal, a female, weighed 10 kgm. As usual after injection of curara (4.5 cc. in 3 doses) the blood pressure fell (from 168 to 50 mm. of mercury, after the first dose) while the limb expanded, the lever going over the top of the drum. After a few minutes the blood pressure rose even beyond the initial level (to 190 mm. of mercury) and remained at this height for a long time. Forty minutes

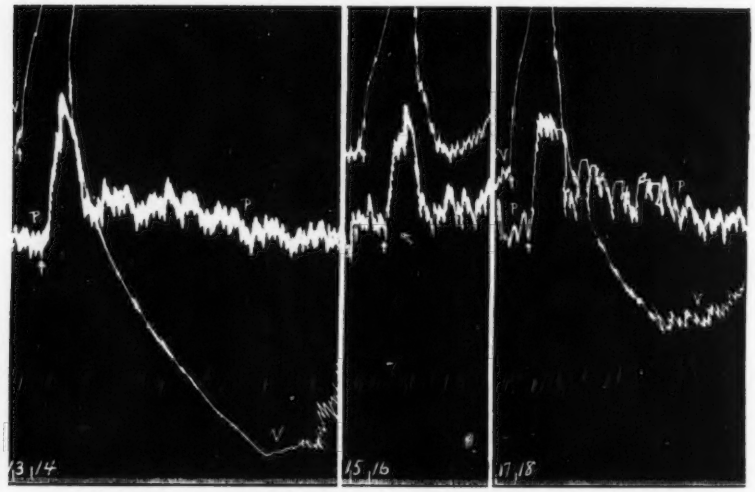


Fig. 16. Tracings from dog 748. Sciatic stimulation; *13–14*, before opening abdomen; *15–16*, after opening abdomen and preparing adrenal veins for clipping (but without clipping); *17–18*, repetition of *15–16*, 6 minutes later. Base-line moved up 40 mm. and the figure then reduced to two-thirds.

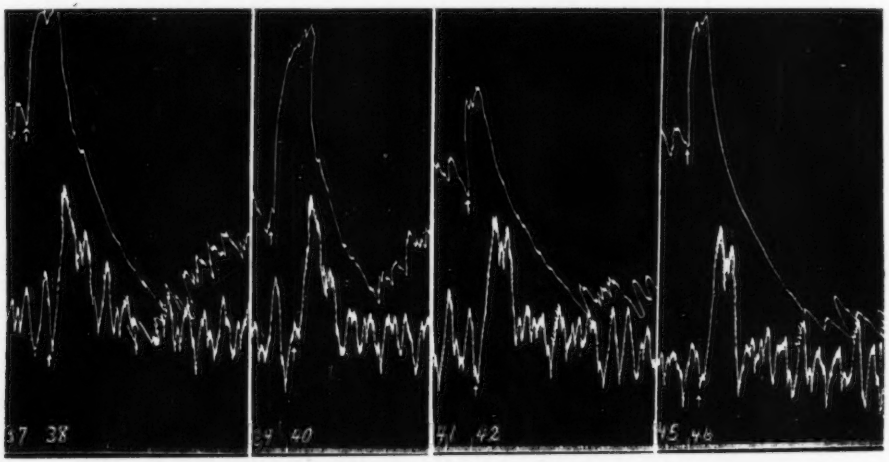


Fig. 17. Tracings from dog 748. Sciatic stimulation; *37–38*, adrenal veins unoccluded; *39–40*, during occlusion of adrenal veins; *41–42*, with adrenal veins still clipped off; *45–46*, after tying off both adrenal veins. Base-line moved up 30 mm. and the figure then reduced to two-thirds.

after the first injection of curara, excellent limb reactions were being obtained on stimulation of the central end of the sciatic (e. g., fig. 16, observations *13* and *14*, and other observations not reproduced). At the end of the portion of the tracing reproduced, it was ascending almost vertically and passed up again through the blood pressure trace. Both adrenal veins were now prepared for clipping. Six minutes thereafter the sciatic was stiumlated with the adrenal veins open (fig. 16, observation *15* and *16*). No positive limb reaction was elicited. After an interval of 6 minutes the stimulation was repeated, and a good reaction was elicited, although not so large as at *13* and *14* (fig. 16, observations *17* and *18*). Both adrenal veins were now clipped and sciatic stimulation repeated without evoking a positive limb reaction. The lever of the recorder never descended nearly to the original lever. The curara action was now wearing off, the dog giving an occasional gasp. The sciatic was again stimulated, the adrenal veins being open, and a large reaction was elicited. More curara (1.5 cc.) was given, and 5 minutes thereafter the sciatic was stimulated with the adrenal veins open. A large positive limb reaction was got. Six minutes later the adrenal veins were clipped, and after the clips had been on for 2 minutes sciatic stimulation gave a small but definite positive limb reaction. On removal of the clips a good reaction occurred. Stimulation of the sciatic with the veins open now yielded a fair reaction, but considerably less than before. Up to this point all the stimulations had been of the same strength and duration (10 seconds, with distance of coils 12 cm.). The nerve was now stimulated for 15 seconds with the veins open, and a good reaction obtained, 6 minutes before observations *37* and *38*, figure 17. To eliminate the possibility that the manipulation necessary for adjusting the clips might have something to do with the suppression of the reaction when the veins were occluded, an observation was made (fig. 17, observations *37* and *38*) in which the maneuvers practised in putting on the clips were gone through, but the veins left open and the sciatic stimulated. The limb reaction was large. Four minutes later the adrenal veins were clipped and stimulation of the nerve repeated, with a good positive result (*39* and *40*, fig. 17). Two minutes later, the adrenal veins being still clipped, the stimulation was repeated, and a limb reaction not inferior to that with the veins open was obtained (*41* and *42*, fig. 17). The adrenal veins were now tied, and 5 minutes thereafter the sciatic was stimulated. The limb reaction was very large, quite as good as with the veins open (*45* and *46*, fig. 17). The right adrenal was then excised, the vein of the left, of course, remaining tied. Sciatic stimulation caused a very large limb reaction, as large as any got with the adrenal veins open. The strength of the stimulus had not been varied, and the duration was 15 seconds in all the observations from the time it was changed from 10 to 15 seconds. After removal of the second adrenal a definite positive reaction was obtained on stimulation of the sciatic.

In this experiment it must be noted that if the observations had not been carried on for a sufficiently long time after the first interference with the adrenals, the conclusion might have been drawn that epinephrin from these glands was indispensable for the reaction. It is clear from the later observations that this conclusion would have been erroneous.

The same point is also illustrated in the experiment on dog 744, a male, weighing 7.9 kgm. At *10* and *11*, figure 18, stimulation of the central end of the sciatic caused a fair limb reaction. The abdomen was now opened and the adrenal veins prepared for clipping. Seven minutes thereafter the sciatic was again stimulated, the stimulus being of the same strength and duration, and the limb reaction was quite as good (fig. 18, observations *12* and *13*). Another experiment (cat 718) has been alluded to, in which after opening the abdomen and preparing the adrenal veins without clipping them, no reaction was at first obtained on sciatic stimulation, although a good one had been got before the operation, and was again obtained afterwards both with the veins clipped and open. This illustrates the fact that the reaction is to some extent capricious, that is, its presence and magnitude are determined by conditions which it is not always easy, or perhaps possible, to control.

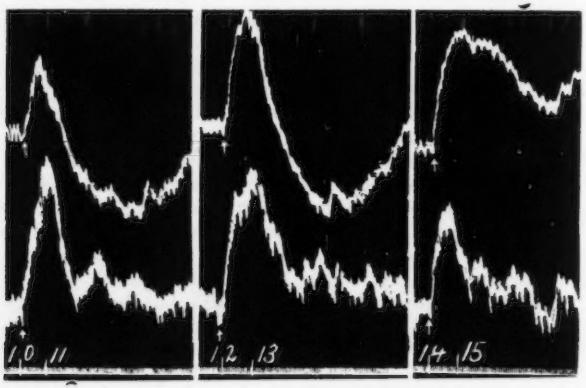


Fig. 18. Tracings from dog 744. Sciatic stimulation; *10–11*, before opening abdomen; *12–13*, after opening abdomen and preparing adrenal veins for clipping (but without clipping); *14–15*, with adrenal veins clipped. Base-line moved up 65 mm. and the figure then reduced to four-fifths.

When stimulation was now repeated (in dog 744) with the adrenal veins clipped the limb reaction was negative (fig. 18, observations *14* and *15*). Removal of the clips was followed by a good reaction. Another pair of observations was then made, each sciatic stimulation being followed by a period of rest of about 6 minutes. Observations *18* and *19* (fig. 19) show the reaction with the veins open, and observations *20* and *21* with the veins clipped. The strength and duration of the stimulus was the same in each case (15 seconds, with coils at 6 cm.). The reaction is quite as strong at *20* and *21* as at *18* and *19*. The limb curve does not ascend quite as high at *20* as at *18*, and the descent does not begin as promptly. But this is merely due to the fact that the blood pressure rise happens to be somewhat less and somewhat more sustained in the second stimulation. A big

reaction followed release of the clips, showing that occlusion had been effective. Ether was now given and the right adrenal excised. Two stimulations were then made, one with the left adrenal vein open and the other with it clipped. Large reactions at least as great with the veins clipped as open occurred in each case. The limb volume curve and the blood pressure curve in this pair of observations rose more abruptly and higher with the vein clipped, no doubt an accidental circumstance. Adrenalin (0.0007 mgm., or 0.00009 mgm. per kgm.) injected in 10 seconds gave a good reaction, and twice that amount a very large one. The limb reaction yielded by sciatic stimulation was larger than the one and smaller than the other of these

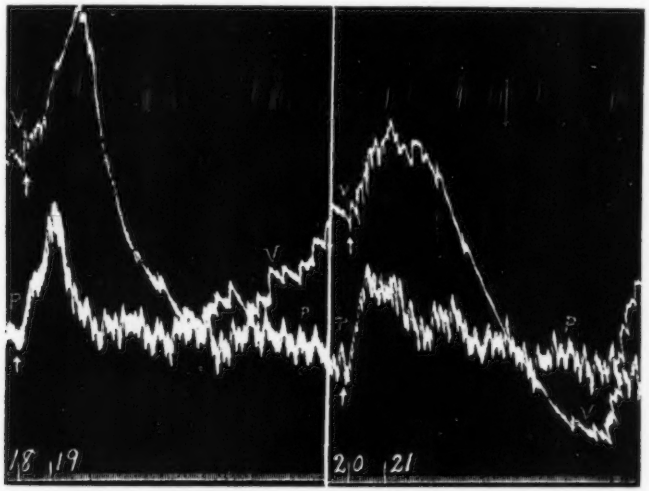


Fig. 19. Tracings from dog 744. Sciatic stimulation; *18–19*, adrenal veins unoccluded; *20–21*, with adrenal veins clipped. Base-line moved up 53 mm. and the figure then reduced to four-fifths.

adrenalin reactions. More curara was now given (2 cc.). Six minutes thereafter, sciatic stimulation with the left adrenal vein open gave only a small limb reaction, whereas 5 minutes later stimulation, of the same strength and duration, with the vein clipped gave a much better limb reaction, accompanied by a somewhat larger elevation of pressure. A subsequent stimulation with the vein open elicited a still larger reaction. The reaction was emerging in the usual way after curara, and there is no reason to believe that it was materially affected by clipping or opening the left adrenal vein. Another stimulation with the vein clipped gave a relatively small reaction, but when it was repeated, the clip having still been left on, a considerably greater reaction was obtained. Removal of the clip was followed by a big reaction. The remaining (left) adrenal was now excised. The

pressure fell somewhat (from 158 mm. to 122 mm. of mercury) and continued to fall slowly, reaching the level of 75 mm., 26 minutes after removal of the second adrenal. Only once or twice thereafter were positive limb reactions elicited by sciatic stimulation. The increases of arterial pressure caused by the stimulation also became insignificant. This certainly had nothing to do with the loss ot epinephrin, since good reactions had been previously obtained with the adrenal vein effectively occluded. But it illustrates the fallacy of assuming that any change which follows removal of the adrenals must be due to loss of the epinephrin output. A very large limb reaction was still caused by the injection of 0.0014 mgm. of adrenalin (0.00018 mgm. per kgm.) in 20 seconds, and a good reaction by the injection of half that amount.

The last experiment (dog 743) from which tracings will be reproduced shows the necessity of testing with more than one strength and duration of stimulus before concluding that interference with the adrenal has abolished the limb reaction. The animal, a male, weighed 7.25 kgm. Sciatic stimulation did not, for some time after injection of curara, yeild a positive limb reaction, but soon a fair reaction began to be obtained. The adrenal veins were prepared for clipping. A fair reaction was now got with the veins open but none with the veins clipped, the volume curve never falling to its initial level. A big reaction, about as large as that caused by injection of 0.003 mgm. (0.0004 mgm. per kgm.) of adrenalin in 10 seconds, was seen on removal of the clips, which had been on for 3 minutes. The sciatic was stimulated 5 minutes later, the adrenal veins now being open, with a positive result. The veins were now clipped, and 2 minutes thereafter stimulation repeated. The result was also positive, although the reaction was not quite as great as with the veins open. Stimulation of the sciatic was again made with the veins open (*35* and *36*, fig. 20). The reaction was probably positive, but as the limb volume curve was falling just before the stimulation, it certainly was not large. All the stimulations hitherto had been for 15 seconds, with 8 cm. between the coils. The adrenal veins were now clipped and the nerve stimulated for 10 seconds (coils 5 cm.) (*37* and *38*, fig. 20). There was no positive reaction. Had the observer stopped here he might have concluded that clipping of the veins had suppressed the reaction. Two minutes later, however, the nerve was stimulated for 15 seconds (coils 8 cm.) the adrenal veins being still clipped, and a large reaction obtained, much larger than with the veins open (*39* and *40*, fig. 20). Removal of the clips caused a good reaction. The adrenals were now excised, and the blood pressure fell from 146 mm. to 66 mm. of mercury. Clipping the adrenal veins had caused no change in the blood pressure in this experiment. Stimulation

of the sciatic gave a negative limb reaction and only a slight rise of pressure. The pressure went on falling steadily to 30 mm. of mercury, and was only raised by injections of Ringer's solution and adrenalin for a very short time.

In other experiments it has been observed not infrequently that when a limb reaction had not yet been obtainable, or had ceased to be evoked by stimulation of the sciatic after elimination of the adrenals, and the

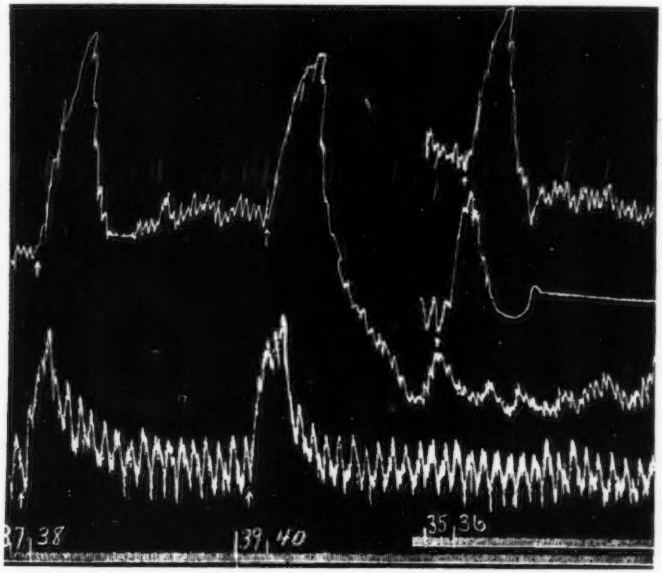


Fig. 20. Tracings from dog 743. Sciatic stimulation; *35–36*, adrenal veins unoccluded; *37–38*, during occlusion of adrenal veins; *39–40*, with adrenal veins still clipped. Base-line moved up 35 mm. at *35–36* and 60 mm. at *37–40*.

blood pressure was low, it could be well obtained if the pressure was raised by injections of Ringer's or salt solution, especially in conjunction with adrenalin. This was the case also in some of the animals which had been subjected to an operation for suppression of the epinephrin output of the adrenals only a relatively short time before the experiment.

For example, in dog 762, a male weighing 6.6 kgm., the right adrenal and half of the left were excised and the nerves of the remnant severed. One week later (the body weight being 6.25 kgm.) an experiment on

the denervated limb was made. Curara (3.5 cc. in 2 doses) caused a permanent drop in the blood pressure from 124 mm. to 55 mm. of mercury. Positive limb reactions were not obtained on stimulation of the sciatic, and the rise of blood pressure was insignificant. Adrenalin (0.0012 mgm., or 0.0002 mgm. per kgm., injected in 15 seconds) gave a good limb reaction. Nearly 2 hours after the first dose of curara the blood pressure was 50 mm. of mercury, and no positive limb reactions had been elicited by sciatic stimulation, although tried for repeatedly. Ringer's solution was now injected (150 cc.), and the blood pressure rose to 87 mm. of mercury. The limb volume curve rose greatly, as very commonly happens after injection of salt solution, a rapid expansion first occurring accompanying the rise of blood pressure, followed by a further long-continued expansion when the blood pressure had ceased to rise. The blood pressure settled at a level of 70 mm. of mercury, which was long maintained, but no positive limb reactions were caused by sciatic stimulation. Only the slightest rise of pressure occurred with each stimulation, accompained by a fair expansion of the limb, but there was no subsequent constriction. Ringer's solution (150 cc.) was again injected, the blood pressure rising to 100 mm. of mercury, and then remaining steady at about 90 mm. Sciatic stimulation now yielded good positive limb reactions in a series of successive observations, a distinct rise of pressure accompanying each stimulation. After a time stimulation of the nerve ceased to elicit reactions, but when the pressure was brought up by injection of another 150 cc. of Ringer's solution to about 110 mm. of mercury, a level long maintained thereafter, positive limb reactions were again got, with good rises of blood pressure. This was 4 hours after the first administration of curara. The remaining portion of the left adrenal was then excised, and thereafter stimulation of the sciatic yielded a positive limb reaction, and stimulation of the right splanchnic in the thorax larger positive reactions, accompanied by good rises of pressure, in successive observations.

The same thing was seen in dog 763, a female, weighing 8.15 kgm. at the time of the adrenal operation (excision of the right and one-third of the left adrenal, with destruction of the remaining medulla by a drill and denervation of the remnant). Two weeks later, the dog now weighing 8.85 kgm., the plethysmograph experiment was done. On injection of the first dose of curara (2 cc.) the blood pressure fell greatly (from 225 mm. to 70 mm. of mercury) and some of the sodium carbonate in the manometer tube entered the circulation. This caused the pressure to shoot up rapidly to 240 mm. or more, and then there was a rapid fall to 60 mm. The pressure remained at about this level, and repeated stimulations of the sciatic failed to yield a positive limb reaction. Adrenalin in the dose of 0.0016 mgm.

(0.0002 mgm. per kgm.) injected in 15 seconds gave an enormous reaction. Ringer's solution was now injected (150 cc.) and the pressure rose to 125 mm. of mercury. The first sciatic stimulation thereafter (9 minutes after injection of the solution) was still negative, but 5 minutes later a positive reaction was obtained, and in 11 minutes more a large reaction, although not nearly as large as that given by 0.0008 mgm. of adrenalin (0.0001 mgm. per kgm.) injected in 15 seconds. The blood pressure now gradually fell to 75 mm. of mercury, and the limb reaction caused by stimulation of the sciatic was no longer positive. More Ringer's solution was injected, the pressure rose, and after a time positive reactions were again elicited by stimulation of the sciatic, and still better by stimulation of the right splanchnic in the thorax. That sciatic stimulation, having become ineffective during a period of low blood pressure, should not immediately become effective when the pressure was raised, is what might be expected. For the centers on which the reaction depends must have time to recover, so that the rise of pressure, for example, may become more prompt and its maximum greater.

The same effect of raising the blood pressure by injection of Ringer's solution was seen in an animal (dog 765) in which the right adrenal had been removed, and then, after an interval of 13 days, one-half of the left adrenal, the remnant being denervated. Although 5 weeks had been allowed to elapse since the second operation, the animal (a female) had not regained its original weight of 7 kgm. by three-quarters of a kilogram when the plethysmograph experiment was made. It seemed otherwise in good condition. But after the initial fall of blood pressure (from 102 mm. to 26 mm. of mercury) due to the injection of curara (2 cc.) the pressure continued low (55 mm. to 60 mm. of mercury). We have the impression that in animals which have been subjected to such adrenal operations, and in which an insufficient interval has been allowed for complete recovery, the blood pressure is more apt than in normal animals to fall to a low level early in an acute experiment.

No positive limb reaction could be elicited by stimulation of the sciatic for 50 minutes after the administration of curara. Ringer's solution was then injected, raising the blood pressure from 51 mm. to 88 mm. of mercury, and the pressure was maintained at or about this level for a considerable time. Twenty minutes after injection of the solution, stimulation of the sciatic began to yield good positive limb reactions, and these continued to be obtained till the pressure had fallen to about 50 mm. of mercury, when the reaction disappeared. Ringer's solution was again injected (an hour after the first injection), the pressure rose from 50 mm. to 76 mm. of mercury, and good typical limb reactions (the best during the experiment) were again elicited repeatedly by stimulation of the sciatic.

Discussion. It is not of course incumbent upon us, and it might be difficult, to explain fully why our results differ from those of some other workers. In cases where interference with the adrenals, or conditions incidentally associated with such interference, seemed to have diminished the facility with which the limb reaction was obtained, we have perhaps allowed a longer time to elapse and made a larger number of tests before concluding that the capacity to yield the reaction had

been permanently lost. We have also tried to eliminate the influence of varying degrees of anesthesia and curarization, which have a powerful influence upon the reaction. Of course we sought the most favorable strength and duration of stimulus, a not unimportant condition in determining the occurrence and especially the magnitude of a response. Further, we have used a large number of animals in which the epinephrin output had been permanently interfered with by operations performed long before the actual experiment, and from which the animals had recovered. When the adrenals have been interfered with in acute experiments the utmost care has been taken to inflict as little damage as possible, the removal of the adrenals being, in our opinion, a major operation of a serious character, not so much perhaps in the surgical as in the physiological sense. It would be absurd to claim that we have been uniformly fortunate and others uniformly unfortunate in this matter. Pearlman and Vincent, for example, used the greatest care, generally contenting themselves with the application of clamps to the adrenal veins, which, in our experience, is the least harmful way of excluding epinephrin. Anrep's procedure of tightening a mass ligature around the glands by pulling on strings drawn out through the flank would seem to involve considerable risk. In two experiments, however, he simply clipped the veins.

Whatever weight may be attached to these suggestions, our tracings, we believe, speak for themselves. They prove beyond doubt that excellent reactions, indicating, according to Anrep, an increased outpouring of epinephrin can be obtained, in the absence of the adrenals, through stimulation of the central end of the sciatic. This shows that some other factor than increased epinephrin output, or indeed some other factor than epinephrin is involved in these reactions, which accordingly cannot be used for resolving the question whether artificial stimulation of the nerve reflexly alters the output or not. That a similar reaction is caused by adrenalin is not doubtful. When it is determined how much adrenalin must be injected at a given rate in order to elicit a reaction of approximately the same magnitude as that given by stimulation of the central end of the sciatic, it is found that the required increase of adrenalin concentration is never many times that maintained by the normal output of epinephrin from the adrenals. Never is there any indication that anything like an outburst of epinephrin produced reflexly by sciatic stimulation would be required to elicit reactions of the magnitude observed, even if it were assumed, which cannot be done, that they are due to epinephrin. In the great

majority of our experiments indeed it was shown that epinephrin was not effectively concerned in the reaction at all. When in rare cases it seemed that it might possibly be concerned there was no evidence that this was due to an increased output of epinephrin per unit of time. Our present results therefore agree with those previously obtained with the pupil of the denervated eye, with our results on the blood pressure method of auto-assay when adrenal blood is collected in a cava pocket, with our results on the denervated heart, and, what is more important because the method is more precise and far freer from possible sources of error than these indirect methods, with our results on the isolated rabbits' intestine segment.

In the light of our demonstration that the limb reaction relied on to prove a reflex increase in the epinephrin output on sciatic stimulation can be obtained perfectly well after elimination of the adrenals, it is unnecessary to discuss at length a recent theoretical disquisition of Opitz (13) on this subject. So far as we can follow his argument, he seeks from a consideration of the time relations of the first and second elevations in the blood pressure curve caused by stimulation of the peripheral end of the splanchnic, to find support for "the view of von Anrep, which refers the final vasoconstriction" of the limb "to a reflex delivery of a greater amount of adrenalin." Almost the whole argument concerns the results of direct stimulation of the splanchnic nerve, and he seems to assume that what happens in this case will happen also when the central end of the sciatic is excited. That, however, is the question to be decided, and we do not believe that anybody is wise enough by looking at blood pressure curves obtained during stimulation of the peripheral end of the splanchnic to determine whether the epinephrin output is increased, diminished or unaffected, by stimulation of the central end of the sciatic. Even when the blood pressure curve taken during stimulation of the sciatic itself is studied it is impossible, we believe, to extract any definite information on this point. There is, however, a straightforward way of determining this, namely, by collecting the adrenal vein blood and assaying its concentration of epinephrin. This method we have adopted, and it is because the results given by it are not in harmony with the conclusions of Anrep and Cannon that we do not accept these conclusions. Opitz says that "for reasons which need not be considered at this time, Stewart and Rogoff claim that the stimulation of the sciatic nerve does not give rise to a greater formation of adrenin per unit of time." The reasons are those just stated, and we believe that if Opitz, and perhaps some other writers, would consider them, it would save much wearisome theorizing on a question which cannot be decided in this way.

That stimulation of the peripheral end of the splanchnic causes an increased output of epinephrin is we believe as certain as any fact in physiology. Yet it is not always possible to find a difference between the blood pressure curve recorded during such stimulation with the adrenals intact and after they have been eliminated. Gley and Quinquaud (14), whose work is not cited by Opitz, have gone so far as to say that there is never any difference, that the double peak is seldom seen in rabbits and only in some dogs and cats, and that when present it

has nothing to do with epinephrin. Their experiments lend no support to the idea that a study of the splanchnic blood pressure curve can show that the epinephrin secretion is reflexly increased by electrical stimulation of the central end of the sciatic. They contradict (15) the alleged proof by Florovsky (16) that reflex excitation of salivary secretion elicited by stimulation of the sciatic is suppressed by removal of the adrenals, and find that this operation does not prevent the reflex. The rise of blood pressure caused by asphyxia is also unaffected by adrenalectomy, and is therefore uninfluenced by the epinephrin secretion of the adrenals (17).

Pearlman and Vincent (6) find that what they consider the characteristic blood pressure curve caused by stimulation of the peripheral end of the splanchnic is altered by elimination of the adrenals. They point out, however, and this we can corroborate, that "the conditions under which the experiment is performed make a very considerable difference in the character of the curve obtained by splanchnic stimulation. There may also be differences characteristic of the different species and of different conditions of the animal." But they state (and the only two curves illustrating the results of stimulation of the central end of the sciatic given in their paper show this clearly) that removal of the adrenals does not affect the blood pressure curve with sciatic stimulation. The conclusion would seem to us fairly obvious, that if the change in the character of the splanchnic curve after elimination of the adrenals proves that increased epinephrin output was responsible for the character of the curve obtained with intact adrenals, the absence of change with sciatic stimulation indicates that increased epinephrin output was not a factor in the sciatic curve obtained with intact adrenals, and that the disappearance of the limb reaction after adrenalectomy is due to some factor other than the loss of a reflexly increased epinephrin output since this ought to affect both curves.

We have always understood that the so-called peculiarities of the splanchnic curve (double rise, and acceleration of the heart coming in at a definite point) were assumed to be associated with direct stimulation of the nerve. We have ourselves shown (18) that acceleration of the heart, after division of the vagi and removal of the stellate ganglia, can be very well obtained by stimulation of the peripheral end of the splanchnic after elimination of the adrenals, so that we cannot accept Anrep's conclusion on this point. Our result has been confirmed by Cannon and Uridil (19), and was also obtained (without denervation of the heart) by Gley and Quinquaud. We have seen splanchnic curves which showed the double rise and were unaffected by removal of the adrenals, and splanchnic curves in which the increased epinephrin output demonstrably played a part. And we can only conclude that while stimulation of the splanchnic can and does increase the epinephrin output, it depends upon the relative importance of this and the other factors which modify the blood-pressure curve after splanchnic stimulation whether the effect of the epinephrin will be plainly visible or not. We thus take up a middle ground between those who claim that the so-called typical splanchnic curve is a phenomenon of considerable constancy, decidedly affected by the epinephrin output, and those who, like Gley and Quinquaud, consider this factor negligible. It ought not to be necessary, but it seems necessary to repeat that when it is proved that electrical stimulation of the splanchnic increases the rate of epinephrin secretion, this does not settle the question of

the effect of electrical stimulation of the sciatic upon the output. That has to be decided for itself, and it helps not at all to remark, as has been done, that it would be a strange thing if a nervous mechanism existed for regulating the epinephrin output and was never thrown into activity. If the epinephrin secretion is reflexly controlled, which is not known, how can anybody know except by trying whether stimulation of the sciatic can evoke the reflex? Plenty of reflexes which are known to exist and can be easily elicited by appropriate excitation of the appropriate receptors cannot be elicited by experimental stimulation of the sciatic.

Opitz seems to think it is necessary for us to prove the truth of what he calls the redistribution hypothesis in order to refute the claim that reflex increase of epinephrin secretion by stimulation of the central end of the sciatic has been demonstrated by such experiments as those of Cannon with the "denervated" heart and with collection of inferior cava blood by a catheter, and the experiments of Anrep on the denervated limb. That is not the case, and we have distinctly stated that it is not incumbent upon us to explain the reactions which have been relied upon for proof of reflex epinephrin secretion through stimulation of this nerve. It is enough to show that suppression of the epinephrin output does not essentially alter the reactions. For example, when Cannon claimed to have furnished the proof by finding acceleration of the "denervated" heart when the adrenals are present but not when they have been removed, we demonstrated that excellent accelerations can be obtained, not only by stimulation of the sciatic but by stimulation of the splanchnic after elimination of the adrenals. Often the acceleration was as great as with the adrenals intact. In our opinion that is enough to show that the reaction in question cannot be relied upon to decide the point at issue. In the case of Cannon's "catheter" experiments we pointed out that the method was quite unsatisfactory, that the curves reproduced did not support the conclusions drawn, and that experiments made by proper methods showed no detectable difference in the rate of epinephrin output whether the sciatic was stimulated or not. We made the suggestion that even if it were granted, which we would by no means do, that Doctor Cannon had found a definitely increased epinephrin concentration during sciatic stimulation in samples of vena cava blood, he could learn nothing of the influence of stimulation upon the rate of output unless he knew how the rate of flow of the blood in the cava might have varied in consequence of the stimulation. We suggested that the vasoconstriction due to sciatic stimulation might cause a slackening in the flow in the portion of the inferior cava in which the adrenal blood mingles with the cava blood, in which case an increased concentration of epinephrin might occur without any change in the rate of output. We did not, and do not believe that Cannon's catheter experiments really demonstrated such an increased concentration. We thought, and think the method incapable of yielding reliable evidence even as to the actual concentration of epinephrin in the cava blood, because, among other reasons, of the high degree of dilution of the adrenal vein blood, as pointed out by Richards and Wood (20). We merely argued that even if an increased concentration, which of course is all that the rabbit segments could give information about, had been detected, it would prove nothing without information as to possible changes in the rate of flow in the inferior cava.

In discussing the effects of splanchnic vasoconstriction on the cava flow we say: "We are not concerned here with compensatory reactions which may occur in other areas, leading for example, as Edwards has shown, to increased flow in the superior cava." This statement seems to Opitz to be "misleading." And he proceeds to say, "If compensatory changes in other regions are to be excluded I fail to see how a redistribution of the blood can be effected." We would not think of calling this statement of Doctor Opitz misleading, since nobody who gives the proper value to words could be for a moment misled by it. It is simply an instance of confusion of thought. We are discussing the possibility of changes in the rate of flow in the upper portion of the inferior cava being caused by vasoconstriction in the splanchnic area under the conditions of Cannon's catheter experiments (21). Cannon draws off blood by a catheter from the inferior cava above the adrenal level, and if the flow in this segment of the inferior cava were to suffer a diminution under the influence of constriction of the splanchnic area, an increased concentration of epinephrin in the blood drawn off would be found, even if the rate of epinephrin output from the adrenals underwent no increase. We point out that a compensatory increase in flow, such as Edwards (22) described in the superior cava, does not concern us for the question under discussion. This is certainly true, since the blood drawn off by the catheter is from the inferior cava, and if the inferior cava flow were cut down to half, the epinephrin concentration in it would be doubled if the output remained unchanged. What happened in the superior cava would not concern us at all, even if the increased flow there was exactly synchronous with the decreased flow in the inferior cava. If this "misleads" Doctor Opitz into thinking that we "exclude" a compensatory change in the superior cava in the sense of denying that it exists, we are sorry, but do not see what we can do about it.

A compensatory increase in flow through the posterior extremities is also emphasized by Edwards as occurring when the splanchnic area is constricted. We hope Doctor Opitz will not find it misleading if we point out that in Cannon's catheter experiments one femoral vein was always tied (in the drug experiments both femorals were tied) and in addition the inferior cava was obstructed by a catheter up to the level at which the chief discharge from the splanchnic area occurs. Will Doctor Opitz assert that under these conditions we are vastly concerned with the compensatory increase of flow described by Edwards through the inferior extremities? We should think the conditions singularly unfavorable for promptly establishing such a largely increased flow by these paths that the great decrease caused by splanchnic vasoconstriction should be at once compensated. There is no reason to doubt that when the vessels of the hind limbs are unobstructed there may be eventually, as Edwards says, a compensatory increase in the flow during splanchnic vasoconstriction. Whether this compensation is so prompt and so perfect that considerable changes in the rate of filling and flow in the anterior segment of the inferior cava may not temporarily occur under the influence of the great alteration of flow in the hepatic and renal veins, and other changes caused by stimulation of the central end of the sciatic, is open to question. Opitz supposes that these compensatory changes constitute a mechanism to ensure "a certain constancy of flow in the cava, so that the heart may not suddenly find itself without an adequate supply of blood." There is good evidence that the venous return to the heart can and does vary within wide limits, and often within a very short period of time.

We do not propose to discuss the measurements of blood flow made by Opitz by means of the "stromuhr." He is in error in supposing that we are ignorant of this valuable statistical work. On the contrary, there are probably few of his papers which we have not read, and with profit, although there are one or two statements which to us are unintelligible.[3] But we doubt very much whether an instrument with the inertia of a stromuhr, one which interposes a not inconsiderable resistance especially in a venous path, and is not suitable for detecting rapid variations, can yield information of much value for the present question. Even the stromuhr measurements, however, seem to show a certain swing in the rate of blood flow in the inferior cava during vasoconstriction of the splanchnic area, an immediate increase in flow, attributed to "squeezing" of blood from the constricted vessels, and then a diminution. If the epinephrin output remains unchanged, as it seems to do during experimental stimulation of the central end of the sciatic, the concentration in the cava blood would be first diminished and then increased. The increase in concentration above the minimum concentration during the period of increased cava flow would be the effective factor in producing reactions, not the absolute concentration. Doctor Opitz states that in a dog weighing about 15 kgm. "the second-volume of the blood stream of the femoral artery equals 0.85 cc." and that "even if considerably increased by the denervation of this part, it does not seem likely that so small a quantity of blood could contain sufficient adrenin to produce vasoconstriction by redistribution in the course of 6 to 8 seconds. This time represents the latency between the beginning of the stimulation of the splanchnic nerve and the appearance of the second summit." Apart altogether from the fact, constantly forgotten by Doctor Opitz, that the question is not whether direct stimulation of the splanchnic can increase the output, which we at any rate cannot doubt, but whether electrical stimulation of the central end of the sciatic can be demonstrated to do so, this statement of Opitz seems to us to have no bearing upon the question. It is the relative change which is the important thing; the absolute amount of blood normally going through a part is of no consequence. The quantity of blood passing through the femoral, be it 0.85 cc. per second, or more or less, is the quantity required to keep up a flow through all the vascular channels of the limb on which epinephrin can act. If Doctor Opitz were to measure the blood flow through the iris of a cat no doubt he would find an immensely smaller absolute flow than through the femoral

[3] For instance, the statement (23) that it is possible for adrenalin injected into the facial vein of a dog to be "swept entirely past the orifice of the hepatic artery" without entering it. We cannot imagine how a hydrodynamic feat of this sort could occur, so long as blood was flowing through the hepatic artery. On the other hand, we readily accept Doctor Opitz's demonstration (24) that the flow in the femoral veins and inferior cava can be easily diminished by increasing the intra-abdominal pressure, as when the diaphragm is caused to descend by exciting the phrenic nerves. And this is one of our reasons for insisting that changes in the respiration or other movements of the animal, e.g., when a non-anesthetized cat tied on a holder is frightened by a barking dog, or the sciatic nerve stimulated strongly in an anesthetized animal, must be considered when blood is collected from the inferior cava by means of a catheter, ostensibly for the purpose of determining changes in the rate of epinephrin output.

artery. And he might consider it "unlikely" that so microscopically small a quantity of blood could contain sufficient epinephrin to affect the pupil of the denervated eye when the rate of flow through the eyeball was changed without change in the epinephrin concentration of the blood coming to it. Nevertheless we have found this to be the case (25). But, since we have shown that Anrep's interpretation of the limb reactions is so far from being the correct one that, as a rule, reactions as large as those elicited with intact adrenals can be obtained after elimination of the epinephrin output of these glands, we need not go further into this matter.

There is one other point, however, in Doctor Opitz's paper which ought to be referred to because it involves a question not so much of physiology as of simple arithmetic, and therefore ought to be capable of being decided. Doctor Cannon (26) argued that because the blood flow through the adrenals is increased when the arterial pressure is raised by stimulation of the central end of the sciatic (and in other conditions which cause increased blood pressure, as shown by us in numerous measurements) "the adrenin would be more *dilute* rather than more concentrated in the adrenal blood," and that "there could be an increased concentration only if the blood flow through the adrenal vessels were retarded." "A faster flow in the inferior cava which might accompany the higher arterial pressure would still further dilute the secreted adrenin. With heightened arterial pressure, therefore, the conditions which would prevail in the inferior cava anterior to the adrenal veins would be highly unfavorable for demonstrating a greater concentration of adrenin." And again, "the critics" (Stewart and Rogoff, to wit) "have not considered that with a rise of pressure the blood would pass more rapidly through the adrenal vessels" (the "critics" have as a matter of fact published the greater part of the data showing this) "and therefore, on the facts of their own views of unvarying adrenal secretion, the higher the pressure the more diluted the adrenin—a condition which renders their argument unsound."

We have tried to point out to Doctor Cannon that if the epinephrin secretion is unchanged while the rate of blood flow through the adrenals is altered, the total amount of epinephrin entering the cava per unit of time is unchanged, whether the adrenal blood flow is doubled or halved. And since the quantity of blood discharged from the adrenal veins into the cava constitutes only a very small fraction of the total cava flow, alterations in the rate of adrenal blood flow cannot sensibly affect the concentration of epinephrin in the cava blood. We should have thought that nothing could be simpler than this little application of the rule of three. But Opitz argues the point over nearly a page of the AMERICAN JOURNAL OF PHYSIOLOGY, and comes to the conclusion that Doctor Cannon is quite right in his contention that if the adrenal blood flow is increased without a corresponding *increase* in the rate of epinephrin secretion, the result will be to diminish the concentration of epinephrin in the blood of the anterior end of the inferior cava. "Since the caval blood flow is not diminished" he (Opitz) "is forced to assume that the caval blood is able to retain its adrenin content . . . . by a greater secretory activity on the part of the adrenal cells in order to charge the larger amount of blood traversing these glands with normal amounts of adrenin. Obviously, the combination of a greater blood flow through the adrenals and retention of their former secretory activity can only lead to a

diminished adrenin content of this blood. Thus it has been shown by Burton-Opitz and Edwards that the venous discharge from the left adrenal body may of raised 40 to 60 per cent above normal by excitation of the splanchnic nerve. In compensation this gland must augment its output of adrenin in an equal measure at least."

We can only repeat that if the rate of epinephrin secretion by the adrenals remains unchanged when the central end of the sciatic is stimulated, an augmentation of the adrenal blood flow will not sensibly alter the concentration of epinephrin in the cava blood, unless the cava flow is changed. If the adrenal flow is doubled and the epinephrin concentration in the adrenal blood reduced to half, this will not affect the concentration in the cava, for twice as much epinephrin-containing blood will enter it as was entering it before. If "in compensation" the gland were "to augment its output in an equal measure at least," so that the concentration in the adrenal blood with the accelerated flow did not fall, the epinephrin concentration in the cava blood would be approximately doubled. If we add to a liter of water 5 cc. of a 2 per cent salt solution, and to another liter of water 10 cc. of a 1 per cent salt solution, we have approximately the same concentration of salt in each case. That is all there is in this "question" and here we propose to leave it.

Of course we have not assumed, "now" or at any other time, "that the stimulation of the splanchnic nerve occasions a considerable slackening of the blood flow in the inferior cava which enables this blood to become charged with *normal* amounts of adrenin." We have suggested that, for instance under the conditions of Cannon's catheter experiments, the cava flow may very likely be decreased during stimulation of the sciatic, which would lead not to "charging" of the cava blood with "normal amounts of adrenin" but to an increased epinephrin concentration in the blood if no change occurred in the rate of secretion of epinephrin.

An increased rate of adrenal blood flow with a corresponding decrease in epinephrin concentration in the adrenal blood would not alter this result. But it should not be forgotten that when the rate of adrenal blood flow is abruptly increased, the rate of secretion of epinephrin remaining unchanged, some temporary increase in the rate at which epinephrin enters the cava ought to occur, owing to the more rapid exit of blood already in the gland and with an epinephrin content corresponding to the previous slower rate of flow.

## CONCLUSION

The constriction of the denervated limb following (passive) dilatation, elicited by stimulation of the central end of the sciatic, which some observers have interpreted as constituting evidence that the rate of output of epinephrin from the adrenals is reflexly increased by such stimulation, has no such significance. For the reaction can be well obtained in acute experiments when these glands have been eliminated by excision, or by clipping or tying the lumbo-adrenal veins between the adrenals and the inferior cava after previously ligating them just before coming to the glands.

The typical limb reaction can also be well obtained in animals which have recovered after operations calculated to suppress the epinephrin output (excision of the right adrenal and a large portion of the left with curetting away the remaining medulla and denervation of the remnant).

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# SOME UNUSUAL APPEARANCES OF NUCLEATED ERYTHROCYTES IN THE CIRCULATION FOLLOWING REPEATED INJECTIONS OF SPLENIC EXTRACT

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During the course of experiments reported elsewhere (1) certain interesting changes in the circulating red blood corpuscles of adult rabbits were observed which we believe warrant a fuller description than was there afforded them. The experiments referred to were designed to determine the effect of repeated injections of splenic extract. The splenic extract was a "protein-free" preparation (2) dissolved in physiological saline solution in suitable amount and injected subcutaneously. Each of four full-grown rabbits, apparently normal at the beginning, was injected with this preparation once daily. During the first seven days the dose was 25 mgm. of the extract per kilogram of body weight; during the second seven days, 50 mgm. per kilogram; and during the next fourteen days, 100 mgm. per kilogram of body weight. At the same time each of two control rabbits received daily subcutaneous injections of physiological saline solution alone. Red blood corpuscle counts were made on each of the rabbits before the injections started and daily or every other day afterwards. When the counts were made blood smears were obtained in the usual manner and subsequently stained with Leishman's stain.

The results of the experiments were in general as follows: Loss of weight in the control animals (8.32 per cent) and in those which received the splenic extract (12.29 per cent); wide and rather characteristic fluctuations in the erythrocyte count in the splenic group with general upward tendency in the counts while there was practically no change in the controls; modification of the resistance to hypotonic salt solution of the red blood corpuscles of the spleen rabbits so that some of them were more resistant and others less resistant than normally; great increase in the number of reticulated erythrocytes in circulation (average

number in the controls 1.5 per cent and in the others 40 per cent); appearance of considerable numbers of nucleated red corpuscles in the circulation of the spleen animals. It is these latter, as they appeared in the smears, that we wish to describe in detail.

During the first two weeks of the experiments increasing numbers of the red corpuscles from the splenic group stained irregularly, presenting an appearance very similar to that described for reticulated erythrocytes

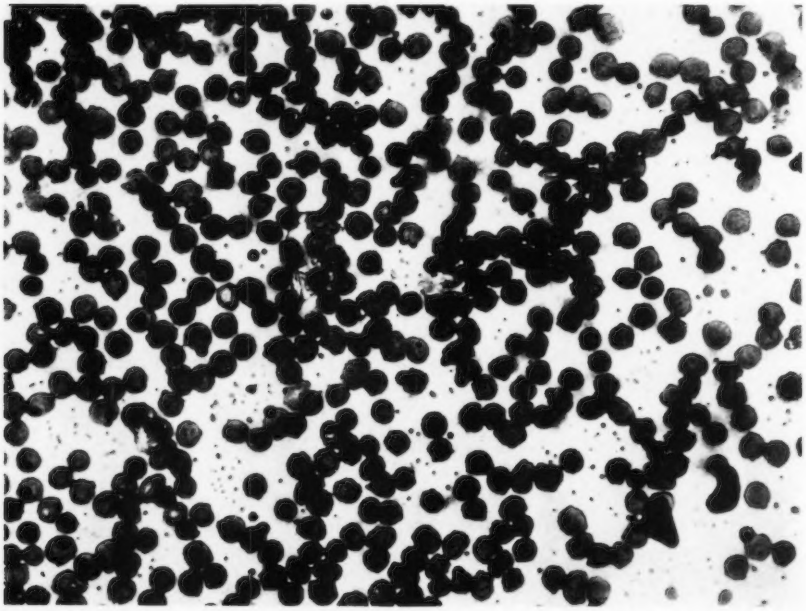

Fig. 1. Photomicrograph of nucleated erythrocytes in circulating blood of adult rabbits after injections of splenic extract. ×450.

vitally stained. Very rarely similar cells were seen in the smears from the controls. During this time no nucleated erythrocytes were found. At no time were nucleated red corpuscles found in the blood of the controls.

On the fourteenth day, which was the time when the dose of splenic extract was changed from 50 to 100 mgm. per kilogram, in each of the smears from two of the splenic animals two nucleated red blood cor-

puscles were found. These cells were larger than the average; the nuclei were relatively large, spherical, centrally placed and not deeply stained.

The following day, the fifteenth from the beginning of the experiments and twenty-four hours after the first 100 mgm. dose, nucleated red corpuscles were found in all the smears from the splenic animals. From this time on nucleated red corpuscles were always present in the

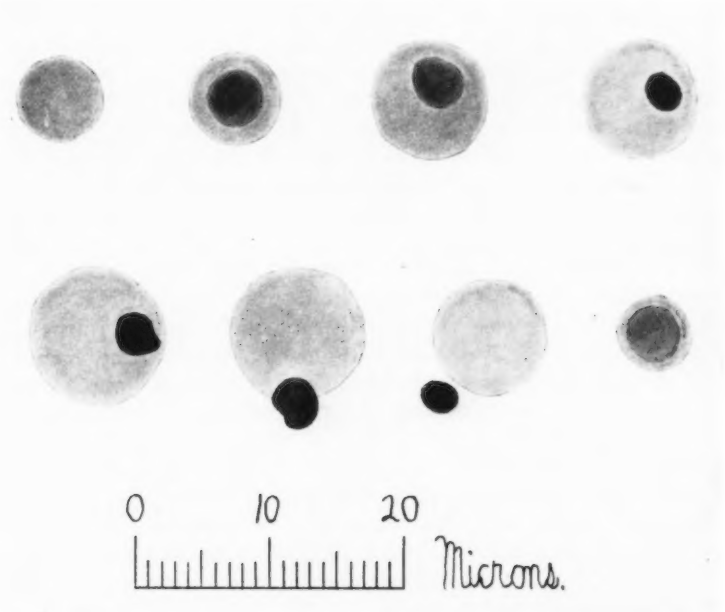


Fig. 2. Drawings of cells seen in the blood smears described showing the nucleus in the various positions.

smears from this group of rabbits and their numbers increased steadily in all to the end of the experiments. In the smears made on the twenty-eighth day from one of these rabbits one-third of the cells were nucleated. Nucleated red corpuscles in all the smears made on this day averaged 13 per cent.

A few of these nucleated cells were similar to those described above as appearing on the fourteenth day, large with relatively large centrally

placed spherical nucleus. Most of them, however, were different. They were even larger in diameter (8 to 10 microns, as compared with a normal diameter of 6 microns) with cytoplasm like the ordinary erythrocyte or less deeply stained. Each contained a single small deeply-stained (pyknotic) nucleus, whose position varied from nearly central to one at the periphery or just outside the cell as if it had just been separated from the cytoplasm. Always the nucleus appeared to be progressing toward the periphery; that is, in the process of separation or extrusion. When the nucleus had reached the periphery of the cell it was impossible to perceive any cytoplasm between it and the cell margin. When the nucleus was separated and lying free just outside of the cell we were unable to detect any cytoplasm about it. In other words there was complete separation of the entire mass of cytoplasm leaving behind a free naked nucleus. The cell from which the nucleus had just been separated was not different in appearance from any of the non-nucleated erythrocytes except for being still somewhat larger.

As the experiments progressed nuclei free of the cells were seen with increasing frequency. Usually these nuclei were small and deeply stained, exactly like the nuclei in the process of separation, still within the cell, and, so far as we were able to detect, entirely free of a surrounding rim of cytoplasm. Now and again other nuclei were seen (twenty-eighth day) which were larger and less deeply stained and about some of these were borders of cytoplasm of varying width. It would seem as if some at least of the nuclei which had become separated from the cytoplasm of a nucleated red corpuscle were able to accumulate about themselves new cytoplasm to make new erythroblasts.

These appearances resemble in many respects the nucleated corpuscles observed by Emmel (3) in the circulating blood of the pig embryo. But in this case we appear to have progression of the nucleus out of the cell rather than separation off of masses of cytoplasm ("cytoplasmic constriction"), inasmuch as the nucleus, reaching the periphery of the cell or taking up its position just outside of the cell, seems to become separated from the entire mass of cytoplasm. However, one could look upon this as a difference in degree rather than in kind.

Our observations are made on fixed and stained preparations so that we did not see the nucleus undergoing these changes; but we believe these appearances represent successive steps in a process of nuclear separation taking place in the active circulation. In addition the possibility of regeneration of new nucleated red corpuscles from the separated nuclei is strongly indicated. Finally we wish to emphasize

that these appearances were observed in smears made from the actively circulating blood of adult rabbits.

## CONCLUSIONS

1. Repeated daily injections of large doses of a splenic extract caused the appearance in the circulation of adult rabbits of large numbers of nucleated red blood corpuscles.

2. The nuclei of many of these cells appeared to be in the process of separation from the cytoplasm.

3. Some at least of these separated nuclei appeared to possess the power of accumulating new cytoplasm for the making of new erythroblasts.

We are greatly indebted to Dr. Ralph F. Shaner for making the illustrations which accompany this article.

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# ABSORPTION FROM THE INTESTINE AND EXCRETION THROUGH THE KIDNEY OF AN UNALTERED COMPLEX PROTEIN SUBSTANCE, TISSUE FIBRINOGEN

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The physiology of intestinal absorption has been for many years, and still is, one of the most interesting questions concerning the animal mechanism. The forces causing the absorption, as well as the exact state of the foods being absorbed, are far from being understood at the present time, although considerable advance has been made especially regarding the preliminary digestion of the food. It remains a much mooted question as to how much of the food eaten is actually digested completely before absorption, or whether there is normally any absorption of undigested whole food compounds. The fact that anaphylaxis is often induced in sensitive individuals from eating certain protein foods points to absorption of undigested material, although we are not yet sure that such effects could not also follow, even with preliminary digestion of the proteins.

That protein absorption can occur in an isolated intestinal loop without any digestion has been shown by Voit and Bauer (1), Heidenhain (2), Friedlander (3), Waymouth Reid (4), Omi (5), Eichhorst (6), Czerny and Latschenberger (7), Cohnheim (8) and many others, although Fischer (9) holds that their conclusions are not justified because they have not entirely excluded the possibility of digestive enzymes being present. According to the last-named author, no absorption can take place unless preceded by digestion or hydrolysis of some sort.

Ascoli (10), by use of precipitin tests, demonstrated egg albumin in the blood and urine of rabbits 1 to 2 hours after it had been injected intravenously while in healthy men it could be demonstrated in the

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blood $1\frac{3}{4}$ hours after eating raw egg white. He found serum albumin always to accompany any other protein in its urinary excretion. Ascoli and Vigano (11) showed that beef and chicken proteins acted similarly to raw egg white in being absorbed. Inouye (12) and Hollande and Gaté (13) also verified by precipitin tests that raw egg white passes into the urine when taken by mouth. Abderhalden, Funk and London (14) were unable to substantiate such findings. Croftan (15), with similar technic, found the egg white in the urine not only when swallowed raw, but also when given by the rectum in rather large amounts. The introduction of a proteolytic enzyme together with the raw egg white prevented the albuminuria.

Wells (16) made use of the more delicate anaphylaxis test for proteins and failed entirely to find the proteins of the food present in the urine in alimentary albuminuria. Instead he found only the blood proteins. However, Van Alstyne and Grant (17), also using the anaphylaxis method, demonstrated egg albumin in the blood of dogs $1\frac{1}{2}$ hours after placing it in a loop of intestine and as long as 24 hours afterward in the urine of the animals. They found that a patient gave tests for raw egg white and milk proteins in his urine after oral ingestion of these substances, but blood proteins were also present.

We find, then, conflicting statements in the literature, although the balance of evidence seems to favor intestinal absorption and kidney excretion of unchanged proteins under experimental conditions which frequently involve a flooding of a portion of the intestinal tract with the protein. Hammarsten (18), after reviewing the work done along these lines, concludes that absorption without digestion is possible under certain conditions, such as *a*, flooding the canal with protein; *b*, greater permeability of the intestinal wall, as in the new-born and sucklings; and *c*, lessened activity of the digestive enzymes, but he believes that normally no such absorption of undigested materials occurs. This represents, probably, the opinion held most generally today. It certainly is rarely considered possible that we may be continually absorbing a part of our protein food in an undigested state; we have such a complete and efficient digestive mechanism to reduce it to simple dialyzable bodies that we consider should pass through the mucosa so much more readily.

We had been working with tissue fibrinogen (thromboplastin) described by one of us (Mills (19)) and had found that it was absorbed unchanged from subcutaneous or intraperitoneal injection, with a consequent shortening of the clotting time of the blood. That the material

was taken up unchanged was inferred, since any digestion or hydrolysis of it *in vitro* very rapidly and completely destroyed its effectiveness in quickening blood clotting. We considered it only a step from subcutaneous or peritoneal absorption to that from the small intestine, so we determined to attempt to throw some additional light on the question of the intestinal absorption of undigested protein substances. Our work was rendered simple by the ease with which tissue fibrinogen could be detected when in its original active condition. Thus, its entrance unchanged into the blood would lessen the clotting time, its excretion in the urine would give a urine which would accelerate the clotting of blood, etc. Working with this sensitive, and yet readily detectable, compound we obtained results which, while very surprising, have been repeatedly verified.

EXPERIMENTAL. We will first detail the results of a few of the experiments on subcutaneous and intraperitoneal injections of the coagulant preliminary to reporting the intestinal work. The clotting time was determined by an exceedingly simple yet accurate method, devised by Peterson and Mills (20). It consists in drawing a drop of blood into a capillary tube of about 1 mm. inside diameter and about 1 inch long, leaving about one-fourth of the tube unfilled; by gently inverting the tube every 30 seconds, one determines the time when the column of blood no longer flows, but remains stationary. This is the end point to be sought. Too frequent inverting of the tube will lengthen the observed clotting time. In order to eliminate a great part of the error due to variations in temperature, the capillary tube is placed in a groove in the palm of the hand so as not to be exposed except for observation. If the bulb of a thermometer be buried in a similar groove of the palm for 3 minutes the temperature will usually be found to be about 34 or 35°C., so that here we have a constant temperature slightly below that of the body. Bürker (21) found a definite relationship to exist between the temperature of the blood being tested and its clotting time. Thus at 13.7°C., he found a clotting time of 18½ minutes and at 34.7°C., 3½ minutes. This latter figure corresponds to the results obtained by our method since the blood here is clotting at 34 to 35°C. This method gives a much shorter clotting time than the test tube method, but the readings are accurate and dependable. Considerable variation will be noted unless care be used in obtaining the blood. The stab with the lance should be of uniform depth so that the tissue juices entering the blood may always be about the same in amount. A little practice readily overcomes this source of error.

*A. Subcutaneous injection of tissue fibrinogen.* The coagulant has been administered in this manner to rats, guinea pigs, rabbits, cats, dogs and men, with results very similar throughout, so there is no need of detailing more than a few of the experiments. It was found that 1 to 2 hours were required for the maximum effect of the coagulant to be in evidence, and that usually the clotting time had returned to, or almost to, normal in 8 to 10 hours after the injection.

*Rat 1*—adult, white.
2:30 p.m.—no treatment—clotting time = 1′ 24″–1′ 20″.
2:50 p.m.—1 cc. coagulant (0.015 gm.) subcutaneously.
4:15 p.m.—clotting time = 33″.
4:50 p.m.—clotting time = 30″.
*Rat 2*—adult, white.
11:00 a.m.—normal clotting time = 1′ 35″.
11:10 a.m.—1 cc. coagulant (0.015 gm.) subcutaneously.
2:00 p.m.—clotting time = 45″.
3:00 p.m.—clotting time = 50″.
4:00 p.m.—clotting time = 54″.
5:00 p.m.—clotting time = 1′ 10″.
6:00 p.m.—clotting time = 1′ 22″.
7:00 p.m.—clotting time = 1′ 30″.
*Dog 1*—male—weight, 7 kilos.
10:30 a.m.—normal clotting time = 2′ 45″.
10:45 a.m.—4 cc. coagulant (0.060 gm.) subcutaneously.
11:30 a.m.—clotting time = 45″.
11:45 a.m.—clotting time = 30″.
12:15 p.m.—clotting time = 20″.
*Man—Jacob Brown*—Syphilitic aneurysm of arch of aorta.
7:30 a.m.—patient lost 800–1000 cc. blood.
11:45 a.m.—clotting time = 3′ 45″–4′ 00″.
12:40 p.m.—2 cc. coagulant (0.030 gm.) subcutaneously.
2:40 p.m.—clotting time = 1′ 45″.
4:25 p.m.—clotting time = 1′ 30″.
8:00 p.m.—clotting time = 4′ 30″.
*Man—Doctor Peterson*—good health.
11:00 a.m.—clotting time = 3′ 00″.
11:46 a.m.—1 cc. coagulant (0.015 gm.) subcutaneously.
1:43 p.m.—clotting time = 2′ 00″–2′ 10″.
4:00 p.m.—clotting time = 1′ 50″–2′ 00″.
6:00 p.m.—clotting time = 1′ 50″.
9:00 p.m.—clotting time = 2′ 10″.

So far as can be determined there are no changes induced in the body by the injection of this coagulant except a shortening of the blood clotting time. It has been used on a considerable number of people

without inducing any general symptoms. Pulse, respiration and blood pressure remain unchanged.

*B. Intraperitoneal injections.* Being desirous of seeing how intense an effect we could produce in animals by injecting the tissue fibrinogen, we tried intraperitoneal injections of large and moderate amounts. It is to be remembered that direct intravenous injections of this substance, even in very small amounts, will produce rapid death by intravascular coagulation of the blood. Intravenous injections of still smaller amounts of very weak solutions quicken the clotting time, but when repeated a number of times, lead to the production of a negative phase, in which the clotting is delayed or absent and the blood fibrinogen greatly reduced. We desired to find whether this same effect could be produced by rapid absorption from the abdominal cavity.

As will be seen below, we obtained only such effects as were produced by subcutaneous administration, except that they came on more quickly, 20 to 60 minutes being sufficient for maximum results. An additional observation, however, was that we now had evidence of an excretion of the coagulant in active form by the kidneys.

*Rat 3*—white, adult.
4:15 p.m.—normal clotting time = 1′ 36″.
4:20 p.m.—1 cc. coagulant (0.015 gm.) intraperitoneally.
4:45 p.m.—clotting time = 55″.
5:10 p.m.—clotting time = 55″.

*Rat 4*—white, adult.
2:10 p.m.—normal clotting time = 1′ 38″.
2:15 p.m.—1 cc. coagulant (0.015 gm.) intraperitoneally.
3:00 p.m.—clotting time = 50″.
3:30 p.m.
4:30 p.m. } clotting time = 20″.
5:30 p.m.—clotting time = 57″.

*Rat 5*—white, adult.
10:30 a.m.—normal clotting time = 1′ 30″.
10:30 a.m.—1 cc. coagulant (0.015 gm.) intraperitoneally.
2:00 p.m.—clotting time = 50″.
3:00 p.m.—clotting time = 32″.
4:00 p.m.—clotting time = 55″.
5:00 p.m.—clotting time = 1′ 18″.
6:00 p.m.—clotting time = 1′ 31″.
7½ hours to return to normal.

*Rabbit 1*—2500 grams.
11:40 a.m.—normal clotting time = 1′ 40″.
11:50 a.m.—1 cc. coagulant (0.015 gm.) intraperitoneally.
12:20 p.m.—clotting time = 40″.
12:55 p.m.—clotting time = 35″.
6:45 p.m.—clotting time = 40″.

*Rabbit 2*—2000 grams.

10:10 a.m.—normal clotting time=1′ 50″.
10:20 a.m.—5 cc. urine obtained by catheterization.
10:30 a.m.—1 cc. coagulant (0.015 gm.) intraperitoneally.
2:30 p.m.—10 cc. urine drawn. Clotting time = 40″.

*Results of tests on these urine samples*

| CITRATED PLASMA | OTHER ADDITION | 1 PER CENT $CaCl_2$ | CLOTTING TIME |
|---|---|---|---|
| cc. | | cc. | |
| 1 | | 0.35 | 4′ 00″ |
| 1 | 0.1 cc. 1st urine sample | 0.35 | 4′ 00″ |
| 1 | 0.2 cc. 1st urine sample | 0.35 | 4′ 30″ |
| 1 | 0.1 cc. 1st urine sample (boiled) | 0.35 | 4′ 40″ |
| 1 | 0.2 cc. 1st urine sample (boiled) | 0.35 | 6′ 35″ |
| 1 | 0.1 cc. 2nd urine sample | 0.35 | 3′ 30″ |
| 1 | 0.2 cc. 2nd urine sample | 0.35 | 3′ 10″ |
| 1 | 0.1 cc. 2nd urine sample (boiled) | 0.35 | 4′ 00″ |
| 1 | 0.2 cc. 2nd urine sample (boiled) | 0.35 | 5′ 30″ |

We see here evidence that some of the coagulant has passed through the kidneys in active form, for whereas the first sample of normal urine slightly retards clotting before boiling, and considerably retards it after boiling, the second sample, drawn four hours after the intraperitoneal injection of 0.015 gram of the coagulant, quite noticeably accelerates the clotting before boiling, but inhibits after boiling as did the normal specimen. We will not attempt to theorize as to how this complex compound gets from the peritoneal cavity into the blood and then out into the urine unchanged but will be content with the demonstration that such a thing undoubtedly does occur. There are no apparent symptoms of any kind on the part of the animal.

*Rabbit 3*—2000 grams.

1:20 p.m.—clotting time = 1′ 45″.
1:30 p.m.—1 cc. coagulant (0.015 gm.) intraperitoneally.
5:00 p.m.—8 cc. urine drawn. Clotting time = 35″.

*Tests on urine sample*

| CITRATED PLASMA | OTHER ADDITION | 1 PER CENT $CaCl_2$ | CLOTTING TIME |
|---|---|---|---|
| cc. | | cc. | |
| 1 | | 0.35 | 4′ 10″ |
| 1 | 0.1 cc. urine | 0.35 | 3′ 05″ |
| 1 | 0.1 cc. urine (boiled) | 0.35 | 5′ 30″ |

Throughout this work we were constantly forced to observe the difficulty encountered in obtaining blood from the injected animals for determining the clotting time. This inability to obtain blood from a stab wound into an ear vein was so extreme at times that it was almost impossible to get an estimate of the clotting time. While under the influence of the injection the animal may fairly be cut to pieces with little hemorrhage, provided no large vessels are severed. Capillary oozing is done away with almost entirely. This should have an important bearing in surgery and in hemorrhages of all sorts.

*C. Intestinal absorption without digestion.* Having observed the passage of the tissue fibrinogen into the blood in an unchanged form from the subcutaneous tissues and peritoneal cavity, we were entirely prepared for the results obtained by intestinal absorption. The main question, of course, was whether the intestinal mucosa would behave like other tissues of the body in permitting passage of the substance, or would require a preliminary digestion either in the lumen of the gut or in its wall. The work reported in the literature so far mainly demonstrates a loss of certain proteins from solutions placed in intestinal loops, without truly proving that the lost substance was to be found in the blood stream. Attempts to do this latter part have always been open to question because of the high concentration of blood proteins and the variations in this concentration that may readily occur normally. We were particularly anxious then to demonstrate the active material in the blood and, if possible, in the urine also. Should this occur, clinical application of considerable value would ensue.

We first investigated absorption from intestinal loops of dogs under ether anesthesia. The clotting time here was obtained by drawing blood directly from the carotid artery into a small test tube and from this filling the small capillary tubes described previously. The carotid cannula was carefully cleaned before each withdrawal of blood and 6 to 8 cc. of blood were allowed to flow before collecting the sample, in order to avoid all chance of getting blood that had lain stagnant in the artery.

*Dog 2*—male, 10 kilos.

2:00 p.m.—Ether anesthesia—open cone method. Loop of upper jejunum prepared and washed free of contents. Sample of urine taken from bladder.
2:30 p.m.—clotting time of blood from carotid = 2′ 30″.
2:35 p.m.—1 cc. coagulant (0.015 gm.) placed in loop.
3:05 p.m.—clotting time = 1′ 10″–1′ 13″.
3:35 p.m.—clotting time = 1′ 50″.
4:05 p.m.—clotting time = 2′ 35″.
4:05 p.m.—Sample of urine taken from bladder.

*Tests on these urine samples*

| CITRATED PLASMA | OTHER ADDITION | 1 PERCENT $CaCl_2$ | CLOTTING TIME |
|---|---|---|---|
| *cc.* | | *cc.* | |
| 1 | | 0.35 | 3′ 00″ |
| 1 | 0.15 cc. 1st urine sample | 0.35 | 3′ 00″–3′ 10″–2′ 50″ |
| 1 | 0.15 cc. 2nd urine sample | 0.35 | 1′ 25″–1′ 45″–1′ 42″ |
| 1 | 0.15 cc. 2nd urine sample (boiled) | 0.35 | 3′ 00″ |

An absorption of the unchanged coagulant is in evidence here, the first observation (after $\frac{1}{2}$ hour) showing the maximum effect, with a diminution and disappearance of this effect $1\frac{1}{2}$ hours after placing the material in the loop. The second urine sample showed that the kidney epithelium was also permeable to the substance, since the urine contained the active unchanged coagulant. The normal urine was inactive and free from protein, while the second sample gave a protein test faintly and possessed a coagulative activity which was destroyed by boiling.

*Dog 3*—female, 8 kilos.

4:15 p.m.—Ether—open cone. Clotting time = 2′ 50″.
4:18 p.m.—3.2 cc. coagulant (0.048 gm.) into washed jejunal loop. Urine sample taken.
4:29 p.m.—clotting time = 1′ 30″.
4:39 p.m.—clotting time = 1′ 30″–1′ 40″.
4:50 p.m.—clotting time = 1′ 55″.
5:00 p.m.—clotting time = 1′ 55″.
5:30 p.m.—clotting time = 2′ 53″.
5:30 p.m. Urine sample taken from bladder.

*Tests on urine samples and loop contents at end of experiment*

| CITRATED PLASMA | OTHER ADDITION | 1 PERCENT $CaCl_2$ | CLOTTING TIME |
|---|---|---|---|
| *cc.* | | *cc.* | |
| 1 | | 0.35 | 4′ 10″ |
| 1 | 0.1 cc. 1st urine sample | 0.35 | 4′ 30″ |
| 1 | 0.1 cc. 2nd urine sample | 0.35 | 3′ 10″ |
| 1 | 0.1 cc. 2nd urine sample (boiled) | 0.35 | 4′ 10″ |
| 1 | 0.1 cc. loop contents | 0.35 | 50″ |
| 1 | 0.1 cc. coagulant sol | 0.35 | 45″ |

We see in this experiment that the maximum effect from absorption is in evidence within 10 minutes of the placing of the substance in the loop, and that the effect is gone in $1\frac{1}{4}$ hours. Again we find the active

substance present in the urine. The one confusing feature here is the fact that the clotting time returned to normal while there was still about 1 cc. of active coagulant unabsorbed in the loop. The last test given in the table above shows that it retained almost its full activity, so digestion in the lumen of the loop must have been slight. There exists the possibility here that the erepsin of the intestinal mucosa became effective during the course of the experiment so that more and more of the absorbing coagulant was digested and its activity destroyed before reaching the blood, while that in the lumen of the gut remained unaffected. The other possibility is that the blood itself builds up a destructive mechanism during this short period. However, this latter is rendered unlikely by the fact that the effect of the coagulant lasts several hours longer after subcutaneous or intraperitoneal administration.

Due to a scarcity of available dogs for further experimentation, two of us volunteered to serve as experimental animals. The clotting time was now taken by using blood from the fingers. The experiments were usually carried out at least 6 hours after the last food was eaten. Substances taken were preceded by one-half pint of cold water and followed immediately by the same amount. This insured quick passage of the material through the stomach and rapid distribution of it over a considerable part of the intestinal tract, due to the active peristalsis set up. Usually one can feel the cold fluid in the intestines within 1 to 2 minutes after swallowing.

*Mills.*

8:10 a.m.—breakfast.
2:50 p.m.—clotting time—(only $H_2O$ since breakfast) = 2′ 30″.
2:55 p.m.—5 cc. coagulant (0.075 gm.) by mouth, accompanied by 1 pint of cold water.
3:35 p.m.—clotting time = 1′ 25″–1′ 35″.
4:13 p.m.—clotting time = 1′ 20″–1′ 30″.
4:40 p.m.—clotting time = 1′ 25″–1′ 30″.

Three samples of urine collected showed no activity in accelerating blood clotting *in vitro*, and were protein free.

*Nakayama.*

7:30 a.m.—breakfast.
2:40 p.m.—only $H_2O$ since breakfast. Clotting time = 2′ 20″–2′ 30″.
2:41 p.m.—5 cc. coagulant (0.075 gm.) by mouth, accompanied by 1 pint of cold water.
3:25 p.m.—clotting time = 55″–60″.
3:50 p.m.—clotting time = 55″–50″.
5:00 p.m.—clotting time = 1′05″–1′20″.
No evidence of coagulant in urine.

In both the above experiments there was observed a very great difference in the ease with which blood was to be obtained from a stab wound of the finger. With Nakayama, especially, considerable difficulty was experienced in obtaining an accurate reading during the height of the reaction.

These striking results showed beyond doubt that the active material was circulating in the blood in considerable amounts. An important question remaining was whether we might not possibly have had a digestion before, and resynthesis of the coagulant after absorption, thus getting the effect observed. To determine this we completely inactivated some of the coagulant by tryptic digestion in neutral solution (using Armour's trypsin). The results are shown below.

*Mills.*

8:10 a.m.—breakfast.
12:30 p.m.—clotting time = 2′ 10″.
12:45 p.m.—5 cc. digested coagulant (0.075 gm.) with 1 pint cold water.
1:20 p.m.—clotting time = 2′ 20″.
1:55 p.m.—clotting time = 2′ 20″.
2:25 p.m.—clotting time = 2′ 20″.

*Nakayama.*

7:30 a.m.—breakfast.
12:30 p.m.—clotting time = 2′ 20″.
12:31 p.m.—5 cc. digested coagulant (0.075 gm.) with 1 pint cold water.
1:00 p.m.—clotting time = 2′ 20″.
1:30 p.m.—clotting time = 2′ 20″.
2:00 p.m.—clotting time = 2′ 30″.

In addition to taking the digested coagulant, a like amount of the boiled substance was swallowed, likewise without effect on the clotting time. Also, water alone is without effect.

The entire lack of effect in these experiments surely establishes that the coagulant is absorbed without change when it produces its effect on the blood. A digestion and resynthesis seems very improbable.

Two more experiments were performed to determine more accurately the onset and duration of the effect in such oral administration of the substance.

*Mills.*

8:00 a.m.—breakfast.
1:42 p.m.—clotting time = 2′ 35″.
1:50 p.m.—1 cc. coagulant (0.015 gm.) by mouth with 1 pint cold water.
2:00 p.m.—clotting time = 2′ 10″.
2:10 p.m.—clotting time = 1′ 50″.

2:20 p.m.—clotting time = 1′ 50″.
2:30 p.m.—clotting time = 1′ 45″.
2:40 p.m.—clotting time = 2′ 00″.
3:00 p.m.—clotting time = 2′ 10″.

*Nakayama.*

7:30 a.m.—breakfast.
1:43 p.m.—clotting time = 2′ 40″.
1:48 p.m.—1 cc. coagulant (0.015 gm.) by mouth with 1 pint cold water.
1:58 p.m.—clotting time = 2′ 35″.
2:08 p.m.—clotting time = 2′ 20″.
2:18 p.m.—clotting time = 2′ 00″.
2:28 p.m.—clotting time = 1′ 50″.
2:38 p.m.—clotting time = 1′ 50″.
2:48 p.m.—clotting time = 1′ 50″.
2:58 p.m.—clotting time = 2′ 10″.

Here we see that the effect is wearing off about 1 hour after administering the substance in 1 cc. amounts, while with 5 cc. the effect was only beginning to disappear in 2 hours. A third experiment was now carried out just after rising in the morning, when absorption should be most rapid.

*Mills*—9–28–22.

6:17 a.m.—nothing since rising—clotting time = 3′ 20″.
6:23 a.m.—1 cc. coagulant (0.015 gm.) by mouth, with ½ pint cold water. The cold water could be felt in the intestines within 1 minute.
6:25½ a.m.—clotting time = 2′ 45″.
6:27½ a.m.—clotting time = 2′ 20″.
6:34 a.m.—clotting time = 3′ 00″.
7:06 a.m.—clotting time = 3′ 15″.

*Mills*—10–8–22.

8:53 a.m.—nothing by mouth since rising — clotting time = 3′ 15″.
8:57 a.m.—3 cc. coagulant + ½ pint ice water by mouth.
8:59½ a.m.—clotting time—1′ 40″.

That there should be evidence of the coagulant entering the blood and being carried to the capillaries of the finger within 2½ minutes certainly forms a strong argument against any digestion and resynthesis in its passage through the mucosa. It is hard to consider it otherwise than as a direct absorption into the blood. Were it to be carried from the intestines by the lymphatics, so early an effect could not have been obtained. It is to be noted here that the effect is largely gone in 10 minutes and entirely gone in 45 minutes. This oral administration of the coagulant has been tried on ten individuals so far and always with effects similar to those given above.

We will finally detail experiments on a partial hemophiliac, showing that, although the clotting time is longer, the same variations are in evidence.

*Raymond Smith*, 21 years of age. Gave a history of profuse and prolonged bleeding with slight injuries since childhood. One brother died at 7 years of age from hemorrhage following the extraction of a tooth. Another died at 5 years from hemorrhage following a cut on the cheek. A third died from an unknown cause on the first day after birth. Other family history was unknown.

9:30 a.m.—no breakfast—clotting time = 14′.
9:36 a.m.—1 cc. coagulant (0.015 gm.) by mouth with 1 pint cold water.
9:46 a.m.—clotting time = 4′ 30″.
10:50 a.m.—clotting time = 8′ 00″.
10:52 a.m.—5 cc. coagulant (0.075 gm.) by mouth with 1 pint of cold water.
11:02 a.m.—clotting time = 7′ 00″.
11:12 a.m.—clotting time = 7′ 30″.
11:22 a.m.—clotting time = 4′ 30″.
11:42 a.m.—clotting time = 6′.
12:00 p.m.—luncheon.
5:00 p.m.—clotting time = 5′ 7′.

The relative effect of the coagulant was the same in the above experiment even though the individual showed evidences of considerable hemophiliac tendencies. The maximum effect of the first dose came on in 10 minutes, while with the second it took 30 minutes. This difference was probably due mainly to a difference in the emptying time of the stomach.

Discussion of results. That a colloidal substance with the complexity of tissue fibrinogen should be able to penetrate animal membranes and tissues with seeming ease and without preliminary digestion or hydrolysis, is somewhat surprising. It is not exactly in accord with the conceptions many people hold as to why the cell and tissue colloids remain in place instead of diffusing everywhere. If it were only the colloidal nature of the tissue compounds that maintained the organization and structure, then why can this complex colloid, tissue fibrinogen, penetrate everywhere with such ease? We know that practically all cells of the body contain this substance and that normally it remains in its place in the organized structure. Yet when injected or administered free it penetrates everywhere. We can scarcely conclude anything else than that the tissue compounds are chemically united in the cells and that it is this, and not limiting membranes, that preserves the integrity of the organization.

The definite and specific test for tissue fibrinogen rendered our results

clear-cut and decisive. There can scarcely exist any reasonable doubt but that we obtained rapid intestinal absorption of the coagulant without any digestion. The extreme rapidity of the absorption, as evidenced in the next to the last experiment, was most astonishing. That such a substance as this could be detected in the blood of the finger tip $2\frac{1}{2}$ minutes after swallowing seems scarcely possible, and yet it occurred. Allowing $\frac{1}{2}$ to 1 minute for the material to reach the small intestines and another minute to be distributed to the finger after entering the blood, we see that the absorption itself must have been surprisingly rapid. Such a result surely precludes any digestive step, judging from our present knowledge of the initial speed of digestive enzymes. Preliminary digestion with trypsin before administering the coagulant also prevents its specific effect on coagulation, so that we are justified in ruling out digestion as a factor in the experiments detailed here.

The significance of this rapid absorption without digestion is difficult to comprehend. How many of the sudden reactions and mysterious attacks of man may be due to this intestinal absorption of unchanged substances? Are we justified in thinking that our foodstuffs must be reduced to amino acids, monosaccharides, etc., before absorption, in the light of the experiments detailed here? Of course, it may be that few substances of so complex a nature would behave as does tissue fibrinogen under these circumstances, still we have no reason to suppose such a thing, since this is a natural protein substance found everywhere in the body.

One fact that requires an explanation and for which we can offer only a tentative one, is the rapid disappearance of the active material from the blood after absorption from the intestines. It will be recalled that in one dog experiment the clotting time had returned almost to normal while there was still some active coagulant in the lumen of intestinal loop. In a recent paper Mills and J. S. Mathews (16) showed that blood serum possessed the property of first increasing the activity of tissue extracts, this increase being later followed by a considerable diminution in the power of the extracts to accelerate blood clotting. We have found proteolytic enzymes to act similarly on tissue fibrinogen, causing a two- to four-fold increase in activity for the first few minutes, followed by a gradual and complete inactivation. May it not perhaps be the enzymes of the blood acting on the absorbed material that so quickly destroys its specific action?

Finally, the clinical application of the facts observed here is bound to

be valuable. Hemorrhages of all kinds (with a possible exception of those in hemophiliacs and patients deeply jaundiced) should be much more easily controlled if these facts are borne in mind and made use of. Where an immediate effect is desired the coagulant may be given by mouth with plenty of water, followed at once by a subcutaneous injection. As the effect of the one wears off, the other will be coming on, so that it should be very useful in the first aid treatment of hemorrhages. Another point of usefulness in the oral administration is to quickly determine the degree of reaction of any individual to the coagulant and thus be able to estimate the dosage needed for subcutaneous injection, as, for example, in surgical operations.

## SUMMARY OF RESULTS

1. Tissue fibrinogen may be absorbed from the subcutaneous tissues, its effects on the clotting time coming on in 1 to 2 hours and lasting usually 8 to 10 hours.

2. It is likewise absorbed from the peritoneal cavity, but more rapidly, the effects beginning about ½ hour after the injection and lasting 6 to 8 hours. Following such injections into rabbits, the active coagulant is to be found in the urine.

3. Absorption from the intestines of the unchanged coagulant occurs with great rapidity. Previous tryptic digestion destroys its action of quickening blood clotting after absorption, so that a digestion and resynthesis of the substance in the experiments quoted is rendered very unlikely.

4. While the coagulant is rapidly absorbed from the intestines, its effect on the clotting time lasts for a relatively short period. The cause for this is not known.

5. Kidney excretion of an active coagulant occurs in dogs after placing the substance in an intestinal loop. It has not been observed in man after oral administration of the material.

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# THE EFFECT OF POTASSIUM CYANIDE ON METABOLISM IN TWO FRESH WATER ARTHROPODS

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The investigations of a large number of workers have shown that KNC in relatively strong concentrations decreases the oxygen consumption and carbon dioxide production of a wide range of animals and plants. The effect of cyanide is similar in such plants as the yeasts and *Aspergillus;* the sponge, *Suberites;* various coelenterates; *Planaria;* different echinoderms and annelids; chitons, bivalve molluscs and nudibranchs; some arthropods, including a crab and a beetle; different vertebrates, fish, frog and mammals; and in addition for various tissues. This work has been summarized by Hyman (1), (2), (3), (4) in her accounts of her own experiments and her review of the literature.

It would seem from this showing that further comment on the subject would be unnecessary if it were not for the negative results obtained by Lund (5), (6) with *Paramecium.* Until the *Paramecium* situation is cleared up and we find whether or not it represents a different type of reaction to the cyanides, each additional bit of data on the subject is of interest. For this reason I wish to record the results from some experiments upon the effect of KNC on carbon dioxide production in nymphs of the may-fly *Heptagenina pulchrella* Walsh and on oxygen consumption in the isopod, *Asellus Communis* Say.

The tests for carbon dioxide production were made with Tashiro's biometer (7) and hence are comparative only. For each experiment nymphs were observed for some fifteen minutes in order to select comparable individuals for experimentation. If two nymphs of similar spontaneous activity were not available, the more active animal was transferred to the cyanide thus loading the experiment against the anticipated result. The preliminary observations were made by an assistant so that the determination was without possible prejudice on the part of the observer.

After exposure to N 0.002 KNC for a definite period, one nymph was

washed in once distilled water and tested in the biometer chamber against a nymph of similar history save only that the preliminary exposure was to water rather than to KNC. The details of the method are described in an earlier paper (8).

Eight such tests in which the cyanide exposure ran from fifteen to twenty-seven minutes showed that in each case the nymph treated with the cyanide was giving off less carbon dioxide following the treatment, than was the control animal.

Seven tests in which the exposure was cut to five minutes resulted in six of the treated nymphs giving less carbon dioxide than did their controls.

Sixteen trials with only one minute treatment with the 0.002 KNC gave the same results in all but four cases.

The possibility that the production of carbon dioxide might have been masked by its absorption by possible traces of the potassium ion not removed by the washing does not seem probable since a few of the nymphs treated for these shorter intervals gave more rather than less carbon dioxide than their untreated controls. The possibility of error on this ground was further removed by treating one individual for twenty-five or more minutes with the cyanide and another wholly similar individual for only one minute and then comparing their rates of carbon dioxide production after washing each as usual. Four such tests were made, in all of which the animal treated for the longer period gave off carbon dioxide less rapidly.

The tests show that even a short exposure to cyanide in relatively strong concentration decreases the rate of carbon dioxide production in treated nymphs when compared with similar non-treated individuals. The five exceptions found may be explained in two ways: first, it will be remembered that the more active nymphs from the preliminary observation were placed in the cyanide when exactly equivalent individuals were not at hand; and, second, the effect may be due to a primary stimulation such as other investigators have reported. Both of these possible factors may have been acting and especially the latter since four of the five exceptions occurred when the cyanide exposure lasted only a minute.

In order to test for a possible preliminary stimulation, experiments were run with a short exposure to a less concentrated solution. Six of the eight nymphs exposed for a few minutes to N 0.0005 KNC showed definite increase in the rate of carbon dioxide production when compared with control nymphs. The data do not show which ion caused

the stimulation. Previous work (9) with the isopod, *Asellus*, indicates that such preliminary stimulation is in part, at least, due to the action of the potassium ion, since with the isopods NaNC was less stimulating than KNC just as $Na^+$ is less stimulating than $K^+$.

The effect of KNC upon oxygen consumption was not tested for may-fly nymphs but this experiment has been made with the common isopod, *Asellus communis*. The oxygen consumption was obtained by the Winkler method and the results are listed in table 1 in terms of cubic centimeters of oxygen per liter of water under standard pressure and temperature conditions.

TABLE 1

*Showing the effect of N 0.001 KNC upon oxygen consumption in Asellus*

| NUMBER ANIMALS | MINUTES EXPOSED | OXYGEN CONSUMPTION IN CUBIC CENTIMETER PER L. | |
|---|---|---|---|
| | | Treated | Control |
| 75 | 30 | 0.28 | 0.45 |
| 100 | 30 | 0.17 | 0.63 |
| 88 | 40 | 0.07 | 1.19 |
| 70 | 30 | 0.23 | 0.71 |

For these experiments, lots of similar isopods were divided into two equal groups each of which was placed in 1100 cc. Erlenmeyer flasks; one in cyanide and the other in water to which they were acclimated. Samples of the water were collected by siphoning, using all due care for the protection of the sample. Two blanks were run for each experiment, one with KNC solution and the other with water. The exposure to the cyanide was sufficient to cause the greater number of the isopods to turn on their backs. This loss of equilibrium is accompanied by considerable muscular activity which, taken with some initial stimulation by the cyanide makes it highly improbable that we are dealing here with a difference in muscular activity only. The majority of the isopods recovered within an hour on being transferred to running water.

The results show that the cyanide decreased markedly the rate of oxygen consumption of the isopods.

These observations on the effect of KNC on *Asellus* and *Heptagenina* introduce nothing new into the cyanide situation but serve to confirm the results obtained with many other forms by a number of workers and to emphasize the disagreement between these and Lund's interpretation of his results with *Paramecium*.

The results are of further interest since in previous work I have found KNC a potent agent in causing reversals in the reaction to water currents of positively rheotactic isopods (10) and in reversing the light reactions of may-fly nymphs (8). There has accumulated a considerable body of evidence supporting the explanation offered in 1912 that these effects are correlated with the depression of general metabolism and the present work gives further support to the conclusion.

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# THE EFFECT OF A RESTRICTED DIET

## I. On Growth

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Having previously determined the effect of a restricted diet on activity, growth and longevity (1), this experiment was planned to demonstrate the effect of under-nourishment, due to a diet restricted to plant life, on the growth, on the ability to reproduce, and on the number and character of the young of the albino rat. It was also intended to carry the experiment long enough to show the effect of the restricted diet not only on successive generations but also on the race. This, however, could not be accomplished as none of the young lived beyond the third generation, and the line of descent became extinct. The results of this experiment will appear in a series of papers.

The material used was from the original stock of albino rats brought in 1903 from the Neurological Laboratories of the University of Chicago. From time to time new strains from other sources had been added. All the rats were, however, more or less closely related due to inbreeding.

Each pair of rats was kept in a separate wire cage $12 \times 12 \times 18$ in size (2). As soon as the rats were old enough to wean they were removed, the sexes separated and placed in reserve cages for future matings. The colony remained throughout the experiment in a large attic room $40 \times 45$ feet in size. This room was well ventilated and was profusely lighted by a sky-light facing the south and containing approximately 300 square feet. The glass in the sky-light was ribbed, which diffused the rays of light. The cages were on the opposite side of the room in the most subdued light. The room was heated and the temperature remained fairly constant. The environment was, therefore, the same for both groups.

The experiment was started in 1911 and was continued without interruption for a little over eight years. From 1912 to October, 1915 the work was carried on jointly by the authors. During the remainder of the time Doctor Card was unfortunately away. Throughout the

entire course of the experiment forty pairs were under continual observation, approximately twenty pairs being used for controls and twenty given the restricted diet. When individuals ceased to reproduce they were usually discarded and the cage restocked with either one or a pair as the case demanded. In restocking the cages of control rats the new individuals were always selected from the control stock. In restocking the cages of the restricted feeders the new material was taken from the restricted group, wherever possible. However, owing to the scarcity of this material, new individuals often had to be introduced from the control stock.

Careful records were kept of the pedigree, the matings, births, deaths, weights, etc., and preserved in card catalog form for ready reference. The matings have been divided into five groups based on the origin of the stock from which the individuals of the pair were taken. The control rats, or omnivorous feeders, have been designated group I. Those matings in which both male and female were selected from the omnivorous stock and had been on an omnivorous diet until the time of mating were placed in group II. When one rat of a pair was taken from the control stock and its mate from the offspring of the restricted feeders, such matings constituted group III. All matings in which both sexes were selected from the young of restricted feeders, and which had been on a restricted diet since birth, formed group IV. Group V was composed of those restricted feeders which toward the end of the experiment were given an omnivorous diet.

The rats were fed and watered at the same hour daily. The amount of food given was always in excess of what they would eat. All were supplied daily with cracked yellow corn and the same vegetables. The diet was varied as much as possible to keep the animals in a healthy condition. The controls, or omnivorous group, had in addition some form of animal protein two or three times a week. The amount of this protein was equal to two or three grams for each rat. Table scraps such as meat, fish, eggs, potato, beans, rice, celery, carrots, cabbage, bread, etc., were used in large quantities. Raw vegetables, such as lettuce and cabbage, were fed once or twice a week. Almonds and walnuts formed a conspicuous part of the diet. Cooked beans when not in sufficient quantities in the table scraps were fed weekly. In feeding the table scraps, that portion given to the restricted group was sorted over carefully to remove as nearly as possible all bits of meat and animal protein. However, tiny bits of meat, meat juices, and the seasoning which involved such substances as butter, milk, animal fat,

eggs, etc., could not be eliminated. The diet of the restricted group, therefore, was not strictly confined to vegetable sources. It corresponded favorably with the diet of the so-called vegetarian.

No attempt was made to study the effect of vitamines and no synthetic food mixtures were given. We have assumed that the requisite vitamines, salts, proteins, carbohydrates and fats would be contained in the average mixed diet of man and have tried to simulate this in the diet given the rats. Since both groups were given the same vegetables and the controls had only protein from animal sources added, we must assume that this addition supplied the animal with something needed which was not gotten in sufficient amount from the vegetable food.

A vast amount of literature dealing with feeding experiments, which has accumulated during the past few years, has shown how the lack of certain food factors affects the growth and health of animals.

The effects on man of the enforced restricted diet in many localities due to the late war have been studied by many authors. Ferguson (3) found that children whose food supply had been reduced to 40 per cent above their basal requirements, were much below the average in height and weight. The diet consisted mainly of bread and potatoes and contained very little eggs, milk, meat, fish and fat. Gibbon and Ferguson (4) state that the children of the working classses in Vienna, whose daily protein intake from animal sources was reduced to 5.3 grams were deficient in growth and weight. The deficiency in weight was more marked than that of stature. Similar findings are reported by Nobécourt (5), who states that the children of Lille, after three years of rations deficient in assimilable protein and fat, showed almost universal retardation in growth and development, both physical and psychic, and that a large percentage between the ages of 10 and 20 had tubercular lesions.

Many other investigators have shown similar results on other animals. A review of these investigations shows: *a*, That often slight modifications of the accessory food factors (vitamins) in the diet of an animal may cause it to deviate from the normal in regard to growth, reproduction and length of life; *b*, that these accessory food factors are found in varying amounts in most foods; *c*, that a number of deficiency diseases are due to a lack of certain food accessories in the diet; *d*, that proteins of animal sources are more easily utilized by an animal than those from plant sources; *e*, that when protein from animal sources constitutes a good portion of the protein intake sufficient growth promoting vitamins

will be supplied for normal growth and reproduction if the total protein intake is not too low; *f*, that animal protein seems to act as a stimulant to greater cell activity, enabling the animal to utilize the food intake to better advantage; *g*, that animals do best and keep in better health if the protein intake is considerably above that required for maintenance; and *h*, that the effect of a restricted diet on the human is similar to that obtained by experimentation on laboratory animals. With these facts before us we are more able to interpret the results of our experiment.

*Comparative maximum weights and life span of parent rats.* The control male rats attained their average maximum weight of 236 grams at the age of 330 days. The control females reached their maximum weight of 116 grams at the age of 382 days. In a previous experiment (1) the males reached their average maximum weight of 290 grams at the age of 440 days and the females, 188 grams at the age of 420 days. The differences in these results may be partly attributed to the fact that the averages in this former experiment are from smaller numbers of individuals and that each rat was segregated and had special care and feeding. Another factor which accounts for the differences was the custom of discarding pairs in this experiment when they ceased to reproduce. The average age at the last litter was for the males 337 days and for the females 358 days. Many were therefore thrown out before they had reached the age of maximum weight. This would lower the average weight and age.

In comparing the maximum weights of groups II and IV of the restricted feeders with those of the control rats, we find a reduction in weight in the males of 35 per cent and in the females from 25 to 28 per cent below the normal. By consulting table 1 it is seen that the greatest loss of weight was in group IV. This is what would be expected, as this group had been subjected to under-nutrition for a longer period than the other groups. It is also seen that the maximum weight of these restricted feeders was attained at a relatively earlier age than in the controls. In each group of restricted feeders the females reached their maximum weight at an older age than the males with the exception of group V, where the reverse is found. Group V also illustrates the established fact that animals which have reached a maximum weight on a restricted diet will grow larger if they are given a stronger diet.

The average age at death or discarding of the control rats is far below the normal age at death. The average span of life for each sex of well-fed rats has been found to be approximately 34 months. The ages as given in table 1, therefore, represent more nearly the average age at

which the parents were discarded after cessation of reproductive activity. In the case of the restricted feeders we find the average ages more nearly coinciding with the age at death as found previously in restricted feeders, where the average at death for the males was 540 days and for the females 570 days. The average ages at birth of last litter for the under-nourished rats was for the males 360 days, and for the females 365 days. A restricted diet, therefore, causes rats to reach their maximum weight, which is far below the normal, at an earlier age, and shortens the span of life.

TABLE 1

*Showing the averages in days of maximum weight in grams, and the average age at death or discarding of the matings in the designated groups*

| | AVERAGE MAXIMUM WEIGHTS AND AGES | | | | | | AVERAGE AT DEATH OR DISCARDING | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Males | | | Females | | | Males | | Females | |
| | Number | Weight | Age | Number | Weight | Age | Number | Age | Number | Age |
| Omnivorous feeders, (controls), Group I | 64 | 236 | 330 | 61 | 166. | 382 | 58 | 548 | 56 | 568 |
| Omnivorous stock, on restricted diet, Group II | 62 | 154 | 310 | 46 | 125 | 377 | 58 | 460 | 46 | 500 |
| Restricted stock, on restricted diet, Group IV | 31 | 153 | 314 | 31 | 119 | 321 | 34 | 484 | 38 | 490 |
| Restricted stock on omnivorous diet, Group V | 18 | 179 | 347 | 16 | 140 | 336 | 17 | 516 | 16 | 509 |

*Comparative growth of the young.* Table 2 gives the average weights of each sex of the omnivorous and restricted diet groups at different ages. The weights from birth to the 35th day are shown in graphic form in figure 1, and from 45 days to 550 days in figure 2. In figure 1 the interval between averages was 3 days, except in the case of the last interval, which was 6 days. In order that the average would represent a larger number of weighings the sum of all weights taken the day before and the day after the given date as well as the weight at the given date was used in determining the average. Each point in the curve therefore represents the average not only of all weighings on the given day, but also those of the preceding and succeeding days. In figure 2 the intervals were 10 days, up to 250 days. In this case the average was computed from the sum of the weights for 10 days, 5 days preceding and

5 days succeeding the given date. From 250 days on the intervals were greatly increased as shown. This accounts for the smoothing out of the curves after 250 days.

By consulting table 2 and figure 1 it is readily seen that there is a difference in the weights of the young at birth. The males of the control group averaged 4.35 grams and the females practically the same

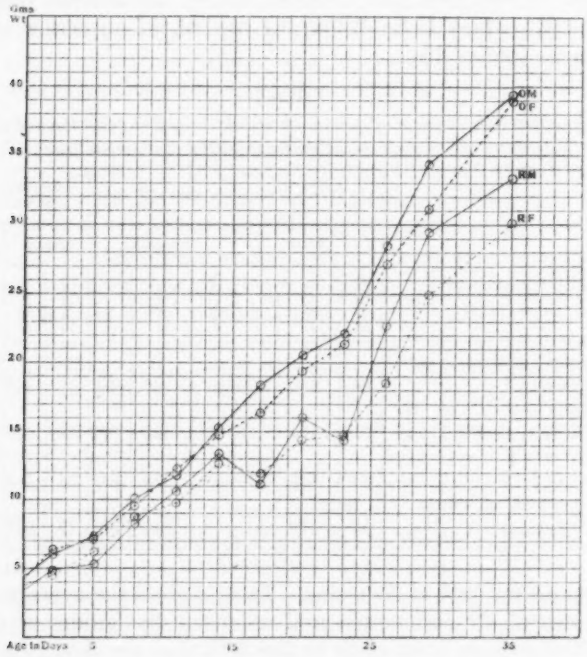


Fig. 1. Graphs showing the average increase in body weight of male, *OM*, and female, *OF*, of the control rats, and of the male, *RM*, and female *RF*, of the restricted group from birth to the age of 35 days.

(4.33 grams). These weights were slightly below the normal as given by other writers. The discrepancy may have been due to differences in food and to the fact that our rats were more or less related and inbred. In the restricted group the young males at birth averaged 0.77 gram or almost 18 per cent below the control males. These males also averaged 0.12 gram less than the restricted females. Comparing the

females of the two groups at birth we find that those of the undernourished group were 0.63 gram, or 14.5 per cent lighter in weight than the control females. It is also noted that in the control rats the males surpass the females throughout life with the exception of the second and eleventh days. At these ages the females were found to weigh slightly more than the males. Both sexes show almost a uniform increase from birth until the age of 105 days, when the uniformity in growth is disturbed. This is doubtless due to the average representing too few individuals.

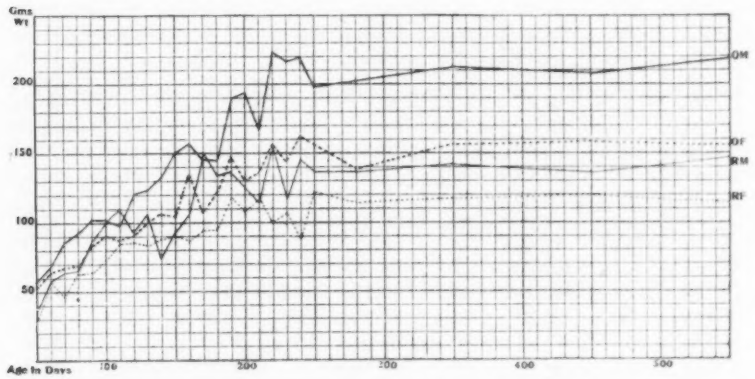


Fig. 2. Graphs showing the average body weight of the male, *OM*, and female, *OF*, of the control rats and of the male, *RM*, and the female, *RF*, of the restricted group between the ages of 50 days and 550 days.

In the restricted group we find that the females were heavier than the males at birth, and at the ages of 5, 8, 17, 23, 55, 135 and 205 days. Both sexes showed much greater irregularity in their curves of growth than those of the controls. The range of variation from the mean of extreme weights at corresponding ages was also very much greater in the restricted group. Most of these fluctuations may be attributed partly *a*, to an insufficient number of weighings to give a correct average; *b*, to the fact that the average represents the weights of all young of the group at the age designated, and thus often includes a whole litter which had failed to make a fair growth and was on the decline. The per cent of mortality was so great in the young of the restricted feeders that this condition was constantly met.

Figure 2 also shows that the effect of the restricted diet on the male

TABLE 2

*Showing the average weight in grams of each sex of the control and restricted groups at the ages indicated*

| AGE IN DAYS | OMNIVOROUS AVERAGE WEIGHTS | | | | RESTRICTED AVERAGE WEIGHTS | | | |
|---|---|---|---|---|---|---|---|---|
| | Males | | Females | | Males | | Females | |
| | Number | Weight | Number | Weight | Number | Weight | Number | Weight |
| Birth | 118 | 4.35 | 110 | 4.33 | 29 | 3.58 | 30 | 3.70 |
| 2 | 129 | 5.98 | 120 | 6.21 | 49 | 4.69 | 76 | 4.49 |
| 5 | 115 | 7.16 | 104 | 7.10 | 18 | 5.18 | 18 | 6.04 |
| 8 | 70 | 10.05 | 75 | 9.47 | 16 | 8.7 | 23 | 8.72 |
| 11 | 65 | 11.6 | 77 | 12.23 | 15 | 10.45 | 18 | 9.74 |
| 14 | 65 | 15.23 | 70 | 14.7 | 18 | 13.22 | 17 | 12.65 |
| 17 | 70 | 18.3 | 65 | 16.3 | 5 | 11.0 | 5 | 11.8 |
| 20 | 58 | 20.56 | 58 | 19.43 | 5 | 16.63 | 4 | 14.25 |
| 23 | 59 | 22.13 | 52 | 21.40 | 9 | 14.30 | 15 | 14.58 |
| 26 | 52 | 28.6 | 41 | 27.2 | 5 | 22.75 | 2 | 18.50 |
| 29 | 55 | 34.5 | 58 | 31.2 | 10 | 29.65 | 8 | 25.90 |
| 35 | 108 | 39.64 | 122 | 39.15 | 16 | 33.5 | 14 | 30.3 |
| 45 | 98 | 57.01 | 96 | 51.24 | 19 | 36.0 | 14 | 29.5 |
| 55 | 91 | 68.03 | 79 | 66.54 | 16 | 58.6 | 17 | 59.7 |
| 65 | 86 | 77.0 | 90 | 68.56 | 19 | 63.2 | 15 | 46.3 |
| 75 | 46 | 82.0 | 63 | 69.1 | 14 | 66.5 | 16 | 63.0 |
| 85 | 44 | 102.86 | 47 | 84.55 | 11 | 86.0 | 15 | 64.0 |
| 95 | 34 | 102.1 | 30 | 90.17 | 11 | 101.0 | 14 | 72.0 |
| 105 | 36 | 97.21 | 39 | 87.93 | 6 | 110.0 | 16 | 85.0 |
| 115 | 46 | 121.53 | 55 | 90.0 | 21 | 92.0 | 11 | 86.0 |
| 125 | 28 | 122.46 | 25 | 100.84 | 14 | 108.0 | 15 | 83.0 |
| 135 | 26 | 132.37 | 22 | 106.78 | 5 | 75.0 | 7 | 89.0 |
| 145 | 21 | 150.5 | 15 | 105.6 | 15 | 96.0 | 20 | 90.0 |
| 155 | 14 | 157.0 | 14 | 135.0 | 9 | 106.0 | 8 | 87.0 |
| 165 | 12 | 145.0 | 20 | 107.0 | 8 | 150.0 | 14 | 95.0 |
| 175 | 17 | 144.0 | 20 | 121.0 | 12 | 133.0 | 11 | 95.0 |
| 185 | 10 | 190.0 | 9 | 149.0 | 14 | 137.0 | 5 | 118.0 |
| 195 | 5 | 193.0 | 7 | 130.0 | 9 | 125.0 | 11 | 97.0 |
| 205 | 10 | 168.0 | 6 | 136.0 | 8 | 115.0 | 4 | 118.0 |
| 215 | 3 | 224.0 | 4 | 157.0 | 8 | 156.0 | 12 | 100.0 |
| 225 | 3 | 217.0 | 6 | 144.0 | 12 | 117.0 | 11 | 107.0 |
| 235 | 6 | 219.0 | 2 | 161.0 | 9 | 145.0 | 3 | 88.0 |
| 245 | 3 | 198.0 | 3 | 156.0 | 10 | 136.0 | 8 | 121.0 |
| 275 | 22 | 202.0 | 20 | 139.0 | 39 | 138.0 | 41 | 114.0 |
| 350 | 63 | 212.0 | 59 | 156.0 | 19 | 141.0 | 18 | 117.0 |
| 450 | 53 | 209.0 | 50 | 159.0 | 19 | 137.0 | 20 | 120.0 |
| 550 | 24 | 219.0 | 41 | 156.0 | 49 | 149.0 | 55 | 115.0 |
| 650 | 10 | 206.0 | 26 | 167.0 | 16 | 151.0 | 20 | 126.0 |
| 750 | 7 | 224.0 | 12 | 162.0 | 4 | 142.0 | 15 | 128.0 |
| 850 | 4 | 250.0 | | | | | | |
| 950 | 5 | 203.0 | 2 | 154.0 | | | | |

was more pronounced than on the female. This is especially noticeable from the age of 250 days on. During this time the restricted males averaged approximately 30 per cent less in weight than the control males, while the restricted females averaged about 23 per cent below the control females. The differences in weight between the two sexes of the restricted feeders is therefore much less than between the two sexes of the controls. The average difference is approximately 25 and 60 grams, respectively.

The above results show that when rats are fed on a diet practically restricted to plant sources they are able to live and grow. This is proof that a sufficiently varied vegetable diet contains the essential factors for growth. But when the rate and amount of growth of rats so fed are compared with that of other rats fed the same vegetables supplemented by additional protein from animal sources we find a marked difference in favor of the omnivorous feeders. This indicates that there is something lacking in the vegetable food which is furnished by the supplementary animal protein. This may be due to additional protein in a form more readily utilized by the animal, or to the part this animal protein may take in acting as a stimulant causing all the cells of the body to become more active and thus making possible a greater and more complete use of the vegetable foods consumed.

## CONCLUSIONS

1. Our restricted diet was not confined to plants, but contained some substances from animal sources, being in this respect closely comparable to the usual so-called "vegetarian" diet of human beings.

2. On our restricted diet the maximum weight of the parent males averaged 35 per cent and the parent females 25 to 28 per cent below the control males and females. The greatest difference was found in the group subjected to the restricted diet longest.

3. The maximum weight was reached at an earlier age by the restricted feeders.

4. The males of the restricted feeders usually reached their maximum weight at an earlier age than the females of the same class.

5. The restricted diet caused a shortening of the span of life.

6. The weights and span of life of restricted feeders were increased on the addition of protein from animal sources.

7. The young of the restricted feeders were below the normal weight at birth. The males were 18 per cent and the females 14.5 per cent below the corresponding sexes of the controls.

8. The effect of the restricted diet on the young became gradually greater until they had reached the age of 250 days, after which little further relative reduction in weight was noticed.

9. The young of the restricted feeders showed greater extremes in weight at given ages than the controls.

10. The young males of the restricted feeders were affected more than the females. The average reductions in weight from the corresponding sexes of the controls were 60 and 25 grams (30 and 23 per cent) respectively.

11. Both sexes of the restricted group averaged less in weight than either sex of the controls.

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# THE VARIATIONS OF THE BLOOD SUGAR OF THE RABBIT THROUGHOUT THE DAY AND THE EFFECT OF THE SUBCUTANEOUS INJECTION OF GLUCOSE

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As a preliminary to a study of the effect of insulin on the blood sugar of the rabbit, it was decided to study the normal variations and the effect of glucose injections. As this paper was being prepared for publication, we were shown, through the kindness of Dr. E. L. Scott, a copy of a paper on the same subject about to be published by Scott and Ford (1). These authors have used the statistical method for the interpretation of their results and, following them, we decided to use the same method for treating our figures. As our results agree rather closely with theirs, it was thought advisable for both papers to be published together.

METHODS. The rabbits used were all in a healthy condition; those in the puerperium and the later stages of pregnancy were rejected. They usually gained in weight during the course of the experiments. When not in use they were kept in cages in which there was always a supply of oats and water. On the morning of the experiment they were taken from these cages and were not fed again until the last sample of blood was taken, although they were allowed access to a dish of water. For the most part they remained sitting quietly in one place, but occasionally they moved from one part of the table to another.

Blood was obtained by cutting the marginal ear vein and allowing the blood to drop into a crucible containing a small amount of potassium oxalate. The rabbits did not, as a rule, resent this, and remained quiet while the sample was being obtained. It might be remarked, in passing, although we do not give figures to justify this statement, that we do not feel that emotion causes any marked change in the blood sugar. Whatever rise there is in a struggling animal we think is due to asphyxia.

The sugar was estimated by the method of Shaffer and Hartmann (2), 1 cc. of blood being used for each determination.

The sugar for injection was weighed out in amounts corresponding to 1 gram and 2 grams per kilogram of body weight. It was dissolved in distilled water and made up to a volume of 20 cc. It was injected subcutaneously in two equal lots in each subscapular region. Following this, samples were taken half-hourly for 2 hours and then hourly for 5 to 7 hours. The statistical methods employed are similar to those of Scott and Ford.

RESULTS: *a. The normal blood sugar.* The total number of observations of normal blood sugar is 157. As part of our object was to see if any variation occurred throughout the day a number of animals were bled at hourly intervals. To these results we have thought it legitimate to add values for animals that had been similarly treated except for the hourly bleeding. These latter in no case amount to more than 20 per cent of the total number. The highest value obtained was 154 mgm. per 100 cc. and the lowest was 83 mgm. The arithmetical mean or average, 116 mgm. per 100 cc. and the median, 115 mgm. per 100 cc., are almost identical. We have expressed the dispersion in two ways: the standard deviation which we obtained from the formula[1] $\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$ where $\sigma$ is the standard deviation, $d$ the individual variations of each value from the mean, and $n$ the number of observations; and the probable error which is 0.6745 $\sigma$, within which range, for a curve following the probability law, half the values should fall.

The standard deviation for this series is 12.2 and the probable error 8.2, within which range lie 69 and 66 per cent of the observations, respectively. This means that there are more values close to this average than one would expect from the probability law. The comments of Scott and Ford apply here except that in our case the majority of the observations lie between 104 and 128 mgm. per 100 cc., the limits of the standard deviation.

The observations have been put in the form of a frequency curve (fig. 1). This will be seen to be of the normal type and fairly symmetrical. The mean, median, range of standard deviation and probable error are shown.

[1] The reason for using $\sqrt{\frac{\Sigma d^2}{n-1}}$ instead of $\sqrt{\frac{\Sigma d^2}{n}}$ is given in the standard works on the subject. See Tuttle—*Theory of Measurements*, 1916, p. 213. This standard deviation, especially when the number of observations is small, is larger than that obtained by the other formula.

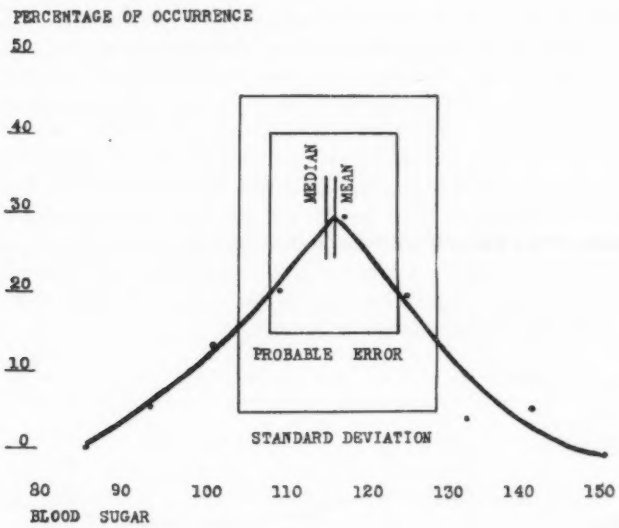


Fig. 1

The values were then tabulated according to the time of day at which they were obtained. These figures are given in table 1 and are plotted in figure 2 which also gives the range of standard deviation. The interesting point is the slight but definite fall between 1 and 3 p.m. followed by a rise to the morning value. On account of the size of the probable error, however, no general rule can be formulated regarding this, although it can be said that a number of rabbits will show it. As

TABLE 1

*Series I. Normal blood sugars throughout the day (mgm. per 100 cc.)*

| Hour | 9–10 | 10–11 | 11–12 | 12–1 | 1–2 | 2–3 | 3–4 | 4–5 |
|---|---|---|---|---|---|---|---|---|
| Number of observations | 34 | 25 | 15 | 15 | 16 | 19 | 17 | 12 |
| Mean | 118 | 118 | 120 | 115 | 112 | 112 | 114 | 117 |
| Median | 121 | 120 | 119 | 114 | 112 | 111 | 113 | 114½ |
| Standard deviation | 18.0 | 15.7 | 10.7 | 8.9 | 7.5 | 8.5 | 13.6 | 14.4 |
| Number within this range, per cent | 76 | 68 | 80 | 60 | 50 | 58 | 71 | 75 |
| Probable error | 12.5 | 10.6 | 7.2 | 6.0 | 5.0 | 5.0 | 9.2 | 9.7 |
| Number within this range, per cent | 68 | 64 | 66 | 60 | 32 | 37 | 47 | 50 |

a matter of fact, about half of those examined do show it. There is, on the whole, a fair degree of constancy throughout the day.

*b. The blood sugar following subcutaneous injections of glucose.* Two series were run. The experiments were in each case begun between 9 and 11 a.m. In series II, the animals (which by the way were the same as used for the normal values) received 0.8 to 1.0 gram of glucose per kilogram body weight, and in series III they received 1.6 to 2.0 grams per kilogram. The results are given in tables 2 and 3. For purposes of comparison with the results of Scott and Ford, an adjusted curve is given in which a number is added algebraically to each mean so that the initial mean is the same as theirs, viz., 118 mgm. per 100 cc. The results have been plotted in figure 3.

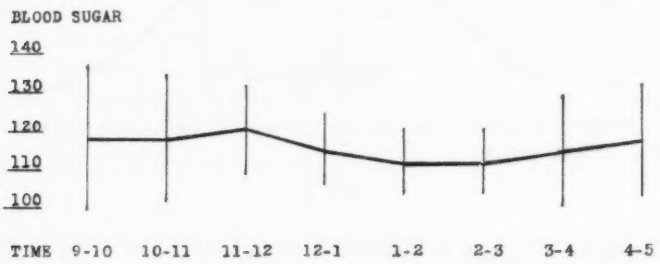


Fig. 2

The standard deviation for the initial values is practically the same as that for the long series (I).

A comparison of the curves for 1 gram dosage reveals a great similarity for the first hour. After that our curve falls much more slowly and does not show any hypoglycemia. At the 3-hour period the lowest individual value is 114 mgm., the average is the same as the initial average. Our series is smaller than that of Scott and Ford and it might be possible that a larger series would show hypoglycemia, but there is nothing in our present series to indicate that this would be the case. We are more inclined to attribute this difference in the curves to the difference in the method of administration of the glucose. Our adjusted curve falls to normal in 3 hours. None of the individual curves reaches the initial value in less than two hours. There is a definite secondary rise beginning in 4 or 5 hours.

Our curves for the 2-gram dose are quite similar to those of Scott and Ford. In neither is there any indication of hypoglycemia. It should

TABLE 2

*Series II. Glucose in dosage 0.8 to 1.0 gram*

| RABBIT NUMBER | DATE | WEIGHT | DOSE PER KILOGRAM | NORMAL | HOURS | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | ½ | 1 | 1½ | 2 | 3 | 4 | 5 |
| | | *kgm.* | | | | | | | | | |
| 47 | 25/7 | 1.97 | 0.8 | 142 | 173 | 146 | 143 | 132 | 114 | 140 | 143 |
| 38 | 6/7 | 3.74 | 0.8 | 121 | 171 | 163 | 163 | 144 | 127 | 131 | 134 |
| 49 | 27/7 | 1.9 | 1.0 | 126 | 181 | 168 | 145 | 136 | 136 | 117 | 139 |
| 50 | 28/7 | 2.4 | 1.0 | 124 | 205 | 193 | 191 | 155 | 134 | 124 | 144 |
| Averages | | | | 128 | 182 | 168 | 160 | 142 | 128 | 128 | 140 |
| Standard deviation | | | | 9.4 | 15.6 | 19.4 | 22.2 | 10.1 | 9.9 | 9.8 | 4.5 |
| Adjusted curve (–10) | | | | 118 | 172 | 158 | 150 | 132 | 118 | 118 | 130 |
| Probable error | | | | 6.3 | 10.5 | 13.1 | 15.0 | 6.9 | 6.7 | 6.6 | 3.1 |

be pointed out that the magnitude of the standard deviation for the periods up to 3 hours points to the extreme variability of the reaction of normal rabbits to the same dose of glucose. In consequence of this it is almost impossible to predict to what height the blood sugar will rise with a given dose of glucose. This has been pointed out by most writers who have studied these curves and the standard deviation merely records this fact in a mathematical way.

TABLE 3

*Series III. Glucose in doses 1.6 to 2.0 gram kgm.*

| RABBIT NUMBER | DATE | WEIGHT | DOSE PER KILOGRAM | NORMAL | HOURS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | ½ | 1 | 1½ | 2 | 3 | 4 | 5 | 6 | 7 |
| | | *kgm.* | | | | | | | | | | | |
| A | 4/6 | 2.39 | 1.6 | 93 | 174 | 161 | 157 | 152 | 115 | 109 | 92 | 113 | 112 |
| 32 | 28/6 | 2.54 | 1.8 | 122 | 186 | 165 | 158 | 154 | 148 | 137 | 149 | 156 | 146 |
| 34 | 27/6 | 2.16 | 1.8 | 122 | 217 | 221 | 218 | 195 | 141 | 141 | 142 | 139 | 136 |
| 44 | 17/7 | 2.24 | 1.8 | 100 | 180 | 146 | 141 | 136 | 130 | 119 | 128 | 128 | |
| 40 | 14/7 | 3.2 | 1.9 | 112 | 216 | 191 | 140 | 122 | 119 | 109 | 113 | 106 | 111 |
| 41 | 12/7 | 2.1 | 1.9 | 122 | 327 | 331 | 262 | 200 | 131 | 112 | 103 | 143 | 138 |
| 45 | 18/7 | 1.38 | 2 | 93 | 182 | 179 | | 164 | 116 | 119 | 116 | 118 | 120 |
| 42 | 13/7 | 2.00 | 2 | 92 | 223 | 187 | 175 | 147 | 116 | 118 | 111 | 119 | 122 |
| 39 | 10/7 | 1.93 | 2 | 127 | 270 | 254 | 216 | 153 | 132 | 137 | 131 | 132 | 124 |
| Averages | | | | 109 | 219 | 204 | 184 | 158 | 128 | 122 | 120 | 128 | 112 |
| Standard deviations | | | | 14.3 | 50.2 | 57.8 | 44.0 | 25.2 | 11.9 | 12.6 | 18.8 | 15.9 | 19.7 |
| Adjusted curve (+9) | | | | 118 | 228 | 213 | 193 | 167 | 137 | 131 | 129 | 137 | 121 |
| Probable error | | | | 9.6 | 33.9 | 39.4 | 29.6 | 17.1 | 8.0 | 8.5 | 12.6 | 10.7 | 13.3 |

Most of the curves do not return to the initial value during the 7 hours for which they were observed. They show a low point in 3 to 5 hours followed by a secondary rise. In one case a value within 10

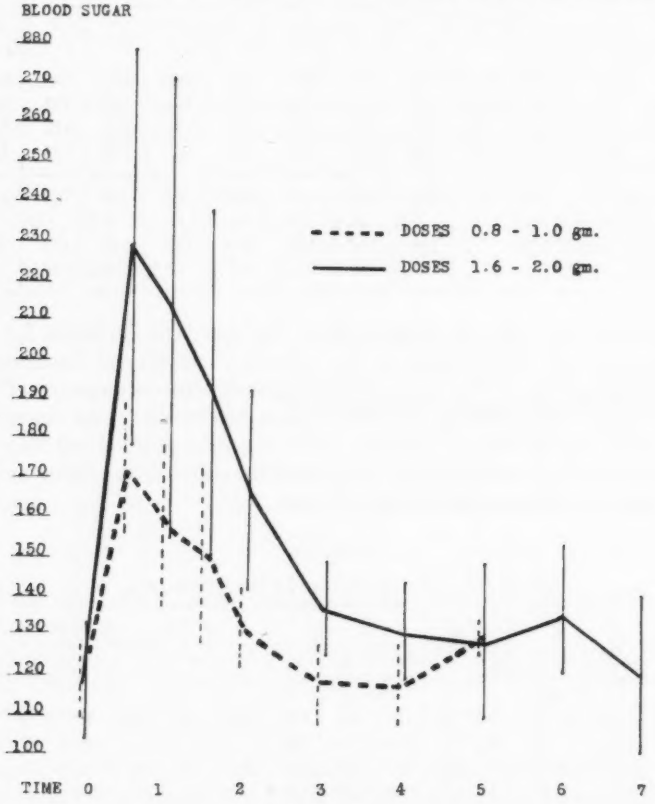


Fig. 3

per cent of the initial was reached in 2 hours. The variation in the time taken to reach the lowest point before the secondary rise is thus quite variable, but not less than 3 hours. Finally after about 7 hours the curve reaches a value which is close to normal.

SUMMARY

1. The concentration of sugar in the venous blood of normal rabbits, determined by the method of Shaffer and Hartmann, has an average of 116 mgm. per 100 cc. of blood, with a "probable error" of 8.2 mgm., within which range 66 per cent of the observations recorded fall.

2. When readings are taken throughout the day a certain proportion of rabbits will be found to have a low blood sugar between 1 and 3 p.m. followed by a return to the morning value.

3. When 1 gram glucose per body weight is injected subcutaneously into rabbits, there is a sharp rise reaching its maximum in half an hour and reaching normal in about 3 hours. This is followed by a smaller secondary rise. Hypoglycemia does not occur.

4. With subcutaneous doses of 2 grams of glucose the rise is much greater but the variation in the height to which the curve rises is also much greater. The curve then falls for 3 to 5 hours, shows a slight secondary rise and returns in about 7 hours to a value near the normal.

In conclusion I should like to express my thanks to Professor Macleod and Dr. J. Satterly for much valuable help.

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# THE CONCENTRATION OF SUGAR IN THE BLOOD OF THE RABBIT DURING INANITION AND AFTER THE INGESTION OF GLUCOSE

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The dog has become, perhaps, the standard laboratory animal for the study of the sugar in the blood, and it may be justifiably so. Before generalizations are made on the basis of such studies it is, however, necessary to remember that the dog is naturally a strict carnivore—in the wild state even an obligate carnivore—and that its mechanism for handling the carbohydrates may be quite different from that of the omnivores and herbivores which ingest much larger quantities of this type of food.

While the rabbit is the most available herbivore which is in common use in our laboratories, it has been rather generally discarded for this type of work because of the feeling that it is very susceptible to emotional hyperglycemia and glycosuria (1), (2). On the other hand we have the statement of Jones (3) that successive determinations of blood sugar upon individual rabbits were constant within the experimental error of the method used for analysis. This statement encouraged us to use the rabbit in the present research. While our results would not warrant us in making the above statement, we believe that they are sufficiently uniform to justify us in drawing certain conclusions which may be of use in the study of carbohydrate metabolism.

METHODS: *a. Chemical.* The method proposed by MacLean (4) for the determination of sugar in blood, modified as noted below, was used throughout the work. The modification consisted in the substitution of phosphotungstic acid as the protein precipitant in place of the one originally described. This necessitated the preparation of a new conversion table, but as no particular difficulties were encountered, and as the reasons for the modification will be discussed elsewhere, the method will not be further described at the present time. The modified method was checked in the usual ways and was found to recover glucose satisfactorily from a solution in water, from blood which

had been incubated at 37°C. until it had lost all reducing power for the copper solution and to which glucose in known amounts had then been added, and from fresh blood to which varying known amounts of glucose had been added.

A few determinations of the glucose in human blood were made in parallel by the modified MacLean method and by the method of Folin and Wu. These determinations gave identical results within the limits of error of the methods.

Duplicates run by the modified method seldom differed by more than 5 mgm. per 100 cc. of blood. With the exception of some half dozen determinations when it was not possible to obtain sufficient blood, or in which one of the duplicates was lost during the analysis, all determinations were run in duplicate and the results reported are the means of the duplicates.

*b. Biological.* All of the rabbits included in the report were in a healthy condition and usually gained in weight while they were under observation. In none of the cases reported was there a significant permanent loss in weight. They were fed once daily on a mixture of grain and green vegetables, usually oats and cabbage. The feeding time was about 9 a.m., except on experimental days, when it was postponed for the rabbits serving as subjects for an experiment until after the last sample was drawn. As the experiments were begun between 10 and 12 in the morning, it will be seen that the rabbits were subjected to an inanition period of at least 24 hours before drawing the initial sample of blood.

When glucose was fed experimentally, it was given in solution by mouth from a metal tipped pipette. Though it was necessary to discard an occasional animal because it would not take the sugar in this way, it was found that in most cases it could be administered without appreciable loss, and we felt that it was much less disturbing to the animal than the use of a stomach tube.

The blood was drawn from the heart and was arterial except in the cases noted in the tables. Occasionally hemorrhage into the pericardium would occur, but no case where this was suspected is included in the present paper.

In studying the alimentary glycemia five samples were obtained, as is usual in clinical practice: first just before administering the glucose, then at the half-hour, hour, and at 2 and 3 hours after feeding. The results of our work would not indicate that any change in these times is desirable, though it might be instructive when it is possible to increase

the number of samples, to extend the time somewhat, especially when large amounts of glucose are fed.

*c. Statistical.* Because of the considerable variation in the concentration of sugar which we found in fasting normal animals, we deemed it desirable to introduce some of the simpler statistical conceptions into our treatment of the results. If the reader is not familiar with the significance of the terms which we have used, he is referred to King (6) or to Yule (7).

EXPERIMENTAL: *a. The sugar present in the blood of the rabbit during inanition.* Because it is so generally believed that the rabbit is not fitted for work of this kind, it was necessary for us to justify its use experimentally before attempting the feeding experiments. This we endeavored to do by determining the sugar in the blood of the rabbits which had been prepared as for the first sample of an experimental series. That is, they were first kept quietly in the laboratory for a few days upon the standard diet. Then after an inanition period of about 24 hours, a sample of blood was obtained and the sugar determined. Only the first sample obtained from a given rabbit in any one day was included in this series. It was, however, thought legitimate to extend the series by adding to it the initial samples obtained in the feeding experiments. These latter constitute about 40 per cent of the entire series.

Though our data bearing upon emotional glycemia in the rabbit are not included in the present paper, it may not be out of place to state that our experience has been that fright is usually, though not always, accompanied by hyperglycemia, and that the degree of hyperglycemia does not necessarily follow the *apparent* emotional disturbance. So far as it is possible to judge, the rabbit does not show as great an increase in glycemia for a given degree of excitement as does the cat. In this connection it should be remarked that the most common emotion in the cat is anger, rather than fright as in the rabbit.

No observations in which the animals did not conform strictly to the standard conditions as outlined above, as well as none in which the rabbit was recorded as being frightened or excited are included in this group. When the work is limited in this way, we have a series of 85 observations upon 27 animals which may be taken as being representative of what may be expected in normal unfrightened rabbits that have been subjected to an inanition period of about 24 hours.

The arithmetical mean for the series is 118 mgm. per 100 cc. of blood. The median is almost the same, 116 mgm. per 100 cc. As a means

On page 523, line 3, of volume 63, Part 3, read:

$$\sqrt{\frac{\Sigma\,(d)^2}{N}} \text{ for } \sqrt{\frac{\Sigma\,(d)^2}{N}}$$

of measuring the dispersion we have adopted the standard deviation as being the most satisfactory. This is represented by the following formula: $\sigma = \sqrt{\frac{\Sigma(d)^2}{N}}$ where $\sigma$ = the standard deviation; $d$ = the arithmetical difference between the individual observations and the mean for the series, and $N$ = the number of observations.

The standard deviation for this series is 21.5 mgm. This gives a range of deviation from 98 to 138 mgm., within which range one would expect the major portion of the observations on any such series to fall. Also, and following from this, one could not safely state that a change in the glycemia, observed during the course of an experiment, was due to the imposed experimental conditions unless it differed from the mean of the control by more than twice the standard deviation, or better, by the sum of the standard deviations of the control series and of a series of animals subjected to conditions similar to those of the experiment. That is, should the range of standard deviation of an experimental series overlap that of the normal series or at most overlap it to any considerable extent, general conclusions could not be drawn, though a modification in a certain direction may occur in a certain proportion of the cases examined.

The results have been plotted in the form of a frequency curve which is shown in figure 1. In the same figure are shown the position of the mean, the median and the range of the standard deviation.

In figure 2 are shown chronologically the concentrations of sugar in the blood of a few individuals which were under observation for a considerable time. This figure makes it clear that it is not safe to depend upon experiments on rabbits, even when the controls are made upon the same animal, unless there has been a considerable number of controls as well as of experiments, and for purposes of generalization these would be preferable if made on a number of individuals.

Since rather wide variations occur in the sugar content of initial samples of blood when the animals have been standardized as carefully as is practicable, we should expect an even greater divergence in their individual responses to experimental conditions which have been imposed upon them. That this is the case is evident when the ranges of standard deviation for the various series shown in figure 3 are compared. From this figure it will also be seen that in a general way the degree of dispersion increases with the degree of modification from the controls. Thus in series 2, 3 and 4 the greatest standard deviation occurs at the time of highest glycemia.

*b. Alimentary glycemia in rabbits.* Altogether four series were run. Series 1 served as a control and was in every way similar to the others except that no sugar was administered. To each rabbit of series 2 was

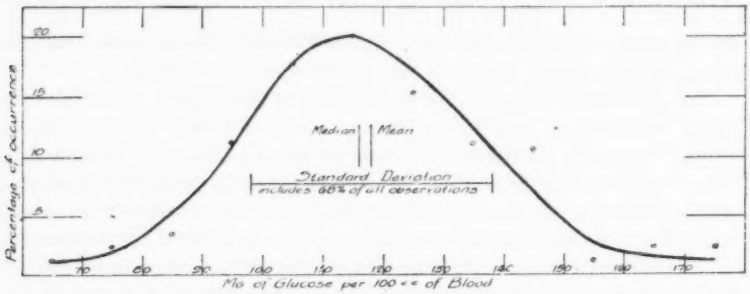


**Fig. 1. Frequency curve showing the distribution of different concentrations of glucose in the blood of rabbits over the range of occurrence.**

given 1 gram of glucose per kilogram of body weight, with a variation in dosage of ± 0.2 gram to each of series 3, 2 grams ± 0.3, and to

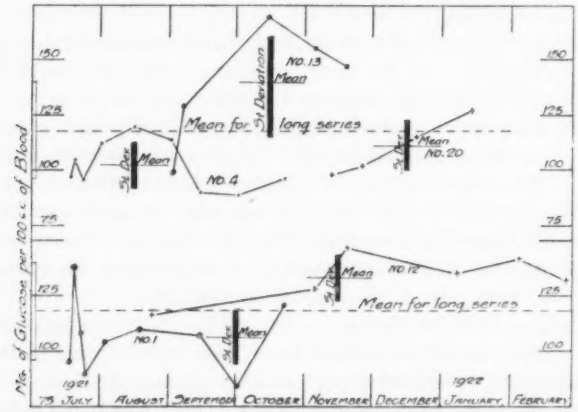


**Fig. 2. Showing chronological variations in the concentration of sugar in the blood of individual rabbits. The standard deviation and mean for the long series are included for comparison.**

each of series 4, 4 grams with a variation of ± 0.8 gram. The means for each period were determined separately for each of the different series, and the standard deviation about each mean calculated. These

TABLE 1

*Concentration of sugar in the blood of normal rabbits after 24 hours inanition*

| EXPERIMENT NUMBER | RABBIT NUMBER | DATE | SUGAR PER 100 CC. OF BLOOD | EXPERIMENT NUMBER | RABBIT NUMBER | DATE | SUGAR PER 100 CC. OF BLOOD | EXPERIMENT NUMBER | RABBIT NUMBER | DATE | SUGAR PER 100 CC. OF BLOOD | EXPERIMENT NUMBER | RABBIT NUMBER | DATE | SUGAR PER 100 CC. OF BLOOD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *1921* | *mgm.* | | | *1921* | *mgm.* | | | *1921* | *mgm.* | | | *1921* | *mgm.* |
| 1 | 1 | 7–18 | 95 | 24 | 1 | 8–3 | 104 | 47 | 1 | 9–14 | 107 | 70 | 19 | 12–20 | 140 |
| | | | | | | | | | | | | | | *1922* | |
| 2 | 2 | 7–18 | 120 | 25 | 2 | 8–5 | 120 | 48 | 4 | 9–14 | 90 | 71 | 21 | 1–7 | 123 |
| 3 | 3 | 7–18 | 116 | 26 | 3 | 8–5 | 128 | 49 | 1 | 10–1 | 84 | 72 | 12 | 1–7 | 135 |
| 4 | 4 | 7–18 | 97 | 27 | 6 | 8–5 | 110 | 50 | 4 | 10–1 | 89 | 73 | 22 | 1–7 | 147 |
| 5 | 5 | 7–18 | 87 | 28 | 6 | 8–8 | 112 | 51 | 17 | 10–1 | 77 | 74 | 21 | 1–14 | 177 |
| 6 | 6 | 7–18 | 96 | 29 | 3 | 8–12 | 132 | 52 | 8 | 10–15 | 127 | 75 | 20 | 1–14 | 127 |
| 7 | 1 | 7–20 | 138 | 30 | 5 | 8–15 | 117 | 53 | 14 | 10–15 | 137 | 76 | 21 | 2–4 | 130 |
| 8 | 3 | 7–20 | 106 | 31 | 4 | 8–16 | 120 | 54 | 13 | 10–15 | 170 | 77 | 12 | 2–4 | 142 |
| 9 | 4 | 7–20 | 105 | 32 | 1 | 8–18 | 110 | 55 | 1 | 10–22 | 120 | 78 | 12 | 2–25 | 132 |
| 10 | 6 | 7–20 | 102 | 33 | 6 | 8–19 | 136 | 56 | 4 | 10–22 | 96 | 79 | 22 | 3–4 | 160 |
| 11 | 1 | 7–23 | 109 | 34 | 9 | 8–24 | 105 | 57 | 17 | 10–22 | 134 | 80 | 23 | 3–4 | 113 |
| 12 | 2 | 7–23 | 105 | 35 | 10 | 8–24 | 110 | 58 | 12 | 11–5 | 128 | 81 | 24 | 3–18 | 147 |
| 13 | 3 | 7–23 | 107 | 36 | 11 | 8–24 | 110 | 59 | 13 | 11–5 | 155 | 82 | 26 | 3–27 | 143 |
| 14 | 4 | 7–23 | 96 | 37 | 12 | 8–24 | 116 | 60 | 18 | 11–12 | 105 | 83 | 26 | 6–3 | 100 |
| 15 | 5 | 7–23 | 105 | 38 | 7 | 8–25 | 120 | 61 | 19 | 11–12 | 119 | 84 | 27 | 6–3 | 114 |
| 16 | 6 | 7–23 | 94 | 39 | 4 | 9–1 | 114 | 62 | 20 | 11–12 | 98 | 85 | 25 | 6–27 | 145 |
| 17 | 1 | 7–25 | 90 | 40 | 13 | 9–2 | 99 | 63 | 12 | 11–19 | 147 | | | | |
| 18 | 2 | 7–25 | 70 | 41 | 15 | 9–2 | 125 | 64 | 8 | 11–19 | 166 | Mean | | | 118 |
| 19 | 5 | 7–25 | 68 | 42 | 17 | 9–2 | 147 | 65 | 13 | 11–19 | 147 | | | | |
| 20 | 2 | 8–1 | 130 | 43 | 5 | 9–6 | 134 | 66 | 19 | 11–26 | 118 | Median | | | 116 |
| 21 | 3 | 8–1 | 116 | 44 | 13 | 9–7 | 129 | 67 | 18 | 11–26 | 118 | | | | |
| 22 | 6 | 8–1 | 105 | 45 | 17 | 9–8 | 103 | 68 | 20 | 11–26 | 102 | Standard deviation... | | | 21.5 |
| 23 | 4 | 8–1 | 112 | 46 | 16 | 9–9 | 120 | 69 | 20 | 12–20 | 115 | | | | |

TABLE 2

*The control series. No sugar was fed*

| EXPERIMENT NUMBER | DATE | RABBIT NUMBER | BODY WEIGHT | TOTAL GLUCOSE FED | GLUCOSE FED PER KILOGRAM OF BODY WEIGHT | SUGAR PER 100 CC. OF BLOOD | | | | | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Initial | Hours after initial | | | | |
| | | | | | | | ½ | 1 | 2 | 3 | |
| | *1921* | | *kgm.* | *grams* | *grams* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | |
| 47 | 9–14 | 1 | 2.00 | — | — | 107 | 105 | 114 | 120 | 120 | |
| 48 | 9–14 | 4 | 1.80 | — | — | 90 | 94 | 94 | 102 | 107 | |
| 67 | 11–26 | 18 | 2.05 | — | — | 118 | 114 | 111 | 98 | 98 | |
| 68 | 11–26 | 20 | 2.50 | — | — | 102 | 105 | 102 | 110 | 110 | |
| | *1922* | | | | | | | | | | |
| 71 | 1–7 | 21 | 2.66 | — | — | 123 | 152 | 140 | 171 | 150 | |
| 83 | 6–3 | 26 | 1.65 | — | — | 100 | 114 | 115 | 116 | 116 | |
| 84 | 6–3 | 27 | 1.55 | — | — | 114 | 127 | 114 | 89 | 125 | |
| Mean | | | | | | 108 | 116 | 113 | 115 | 118 | |
| Standard deviation | | | | | | ±10.6 | ±17.6 | ±13.2 | ±24.8 | ±15.4 | |
| Adjusted mean; correction +10 | | | | | | 118 | 126 | 123 | 125 | 128 | |

TABLE 3

*Sugar in the blood of rabbits after feeding 1 gm. (±0.2) of glucose per kilo of body weight*

| EXPERIMENT NUMBER | DATE | RABBIT NUMBER | BODY WEIGHT | TOTAL GLUCOSE FED | GLUCOSE FED PER KILOGRAM OF BODY WEIGHT | SUGAR PER 100 CC. OF BLOOD | | | | | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Initial | Hours after feeding | | | | |
| | | | | | | | ½ | 1 | 2 | 3 | |
| | *1921* | | *kgm.* | *gms.* | *gms.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | |
| 38A | 8–29 | 1 | 2.05 | 2.0 | 1.0 | 130 | 234 | 159 | 125[1] | 114 | [1] Venous blood |
| 58 | 11–5 | 12 | 1.43 | 1.3 | 0.9 | 128 | 191 | 152 | 127 | 140 | |
| 59 | 11–5 | 13 | 2.22 | 2.0 | 0.9 | 155 | 250 | 198 | 177 | 128 | |
| 60 | 11–12 | 18 | 2.20 | 2.0 | 0.9 | 105 | 125 | 125 | 100 | 96 | |
| 61 | 11–12 | 19 | 1.96 | 2.0 | 1.0 | 119 | 164 | 127 | 98 | 90 | |
| 62 | 11–12 | 20 | 2.40 | 2.4 | 1.0 | 98 | 145 | 141 | 100 | 110 | |
| 70 | 12–20 | 19 | 1.70 | 2.0 | 1.2 | 140 | 173 | 195 | 158 | 116 | |
| Mean | | | | | | 125 | 183 | 157 | 126 | 113 | |
| Standard deviation | | | | | | ±18.2 | ±42.1 | ±27.6 | ±28.7 | ±16.0 | |
| Adjusted mean; correction −7 | | | | | | 118 | 176 | 150 | 119 | 106 | |

TABLE 4

*Sugar in the blood of rabbits after feeding 2 grams (±.03) of glucose per kilo of body weight*

| EXPERIMENT NUMBER | DATE | RABBIT NUMBER | BODY WEIGHT | TOTAL GLUCOSE FED | GLUCOSE FED PER KILOGRAM OF BODY WEIGHT | SUGAR PER 100 CC. OF BLOOD | | | | | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Initial | Hours after feeding | | | | |
| | | | | | | | ½ | 1 | 2 | 3 | |
| | *1921* | | *kgm.* | *grams* | *grams* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | |
| 23 | 8–1 | 4 | 1.67 | 3.0± | 1.8± | 112 | 202 | 168 | 136 | 112 | |
| 24 | 8–3 | 1 | 1.90 | 4.0 | 2.1 | 104 | 220 | 201 | 186 | 133 | |
| 28A | 8–10 | 2 | 0.96 | 2.0 | 2.1 | 122 | 218 | 163 | 195 | 161[1] | [1] Venous blood |
| 30 | 8–15 | 5 | 0.77 | 1.5 | 2.0 | 117 | 207 | 163 | 104 | 110 | |
| 31 | 8–16 | 4 | 1.72 | 4.0 | 2.3 | 120 | 239 | 205 | 184 | 129[1] | [1] Venous blood |
| 32 | 8–18 | 1 | 1.77 | 3.5 | 2.0 | 110 | 129 | 138 | 175[1] | 139 | [1] Venous blood |
| 39 | 9–1 | 4 | 1.78 | 4.0 | 2.2 | 114 | 200 | 218[1] | 143[1] | 99 | [1] Venous blood |
| 44 | 9–7 | 13 | 1.80 | 4.0 | 2.2 | 129 | 163 | 187 | 140 | 120[1] | [1] Venous blood |
| 45 | 9–8 | 17 | 2.10 | 4.0 | 1.9 | 103 | 147 | 182 | 168 | 175[1] | [1] Several punctures were necessary; rabbit became very excited |
| 50 | 10–1 | 4 | 1.90 | 4.0 | 2.1 | 89 | 140 | 200 | 96 | 136[1] | [1] Rabbit became very much excited |
| 51 | 10–1 | 17 | 2.35 | 5.0 | 2.1 | 77 | 176 | 164 | 154 | 159 | |
| 54 | 10–15 | 13 | 2.16 | 5.0 | 2.3 | 170 | 312 | 280 | 132 | 110 | |
| | *1922* | | | | | | | | | | |
| 78 | 2–25 | 12 | 1.85 | 4.0 | 2.2 | 132 | 216 | 173 | 140 | 125 | |
| Mean | | | | | | 115 | 198 | 187 | 150 | 131 | |
| Standard deviation | | | | | | ±21.5 | ±46.8 | ±34.2 | ±29.4 | ±21.7 | |
| Adjusted mean; correction +3 | | | | | | 118 | 201 | 190 | 153 | 134 | |

TABLE 5

*Sugar in the blood of rabbits after feeding 4 grams (±0.8) of glucose per kilo of body weight*

| EXPERIMENT NUMBER | DATE | RABBIT NUMBER | BODY WEIGHT | TOTAL GLUCOSE FED | GLUCOSE FED PER KILOGRAM OF BODY WEIGHT | SUGAR PER 100 CC. OF BLOOD | | | | | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Initial | Hours after feeding | | | | |
| | | | | | | | ½ | 1 | 2 | 3 | |
| | *1921* | | *kgm.* | *grams* | *grams* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | |
| 53 | 10–15 | 14 | 1.37 | 5.0 | 3.6 | 137 | 187 | 293 | 173 | 180 | |
| 63 | 11–19 | 12 | 1.46 | 5.0 | 3.4 | 145 | 220 | 218 | 185[1] | 152 | [1] Some difficulty in obtaining the blood; this sample not obtained; value given mean of 1st and 3rd hour |
| 65 | 11–19 | 13 | 2.44 | 9.0 | 3.7 | 147 | 253 | 267 | 207[1] | 147 | [1] Rabbit became excited; 2nd hour sample not taken; value given is the mean of 1st and 3rd hour samples |
| 66 | 11–26 | 19 | 2.00 | 8.0 | 4.0 | 118 | 222 | 189 | 156 | 117 | |
| 69 | 12–20 | 20 | 2.33 | 7.5 | 3.2 | 115 | 164 | 192 | 150 | 134 | |
| | *1922* | | | | | | | | | | |
| 72 | 1–7 | 12 | 1.53 | 5.0 | 3.3 | 135 | 294 | 282 | 258 | 152 | |
| 76 | 2–4 | 21 | 2.98 | 10.5 | 3.5 | 130 | 263 | 254 | 167 | 170[1] | [1] Some difficulty in obtaining this sample; rabbit apparently not excited, but several punctures necessary |
| 80A | 3–18 | 24 | 2.90 | 10.0 | 3.5 | 147 | 204 | 238 | 184 | 197 | |
| 81 | 3–18 | 25 | 1.40 | 6.0 | 4.2 | 140 | 200 | 204 | 150 | 120 | |
| 82 | 3–27 | 26 | 1.66 | 6.5 | 3.9 | 143 | 204 | 211 | 204 | 152 | |
| Mean | | | | | | 136 | 221 | 235 | 183 | 152 | |
| Standard deviation | | | | | | ±10.9 | ±36.8 | ±35.7 | ±31.5 | ±23.9 | |
| Adjusted mean; correction −18 | | | | | | 118 | 203 | 217 | 165 | 134 | |

values are shown in tables 2 to 5. The adjusted means shown in the last line of each of the tables were obtained by adding algebraically to each time mean of the series an amount which would make the mean for the initial period equal to that of the control series shown in table 1, i.e., 118 mgm. per 100 cc. The values so obtained were plotted and the curves resulting are shown in figure 3. In this way the curves all originate in the same point, and the changes due to the feeding of the sugar are not confused by slight variations in the initial samples.

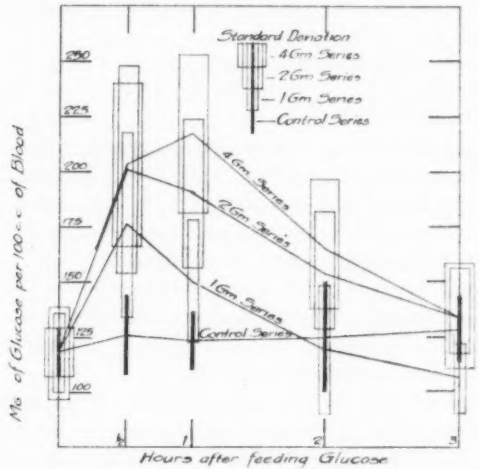


Fig. 3. Showing the effect of feeding various amounts of glucose upon the concentration of this sugar in the blood of rabbits.

A study of figure 3 reveals that: *1, a.* The range of standard deviations for the initial observations of the different series is in no case in excess of that for the long series. The initial condition of the rabbits may then be presumed to be at least as uniform in each of the experimental series as it was in the longer series, and so the different series may be considered as being fairly comparable among themselves.

*b.* The control curve lies reasonably near the horizontal, and none of its ranges of standard deviation is excessive, nor do any of them exceed the ranges for the other series at the respective intervals; so that it may be presumed that the operation of drawing the blood is not a factor in the modifications which occur in the experimental series, and that it is legitimate to take this series as a basis for comparison.

*2.* At the end of the first half hour: *a.* The position of the mean for the 1-gram series is well above that of the controls, and while the range of standard deviation overlaps that of the control series to some extent, it lies wholly above the mean for that series and extends far above its upper limit of deviation. There would seem then to be little question that the administration of this amount of glucose will in most cases cause a considerable rise in the concentration of sugar in the blood at the end of half an hour; though there will be a few cases, say one in five, which would give values of the same order of magnitude as would be expected in fasting individuals.

*b.* The position of the means for the 2- and the 4-gram series indicates a still greater rise, and here even the lower limits of the ranges of standard deviation are well above the upper limit of that of the control, i.e., there is no overlapping. From this we would expect to find very few animals in either series giving values which could be confused with those given by control animals. On the other hand, one could not so surely distinguish between these two series and the one gram series, as here there is considerable overlapping.

*c.* The concentration of sugar in the blood drawn at this time would give no information as to whether 2 or 4 grams of glucose were fed, as in these cases the overlapping of the ranges of deviation is complete and the means are practically identical.

*3.* At the end of an hour the mean for the 1-gram series has fallen markedly, and its range of standard deviation now extends below that for the control series. We would now hardly expect more than two observations in three to be distinguishably higher than the controls. The means for the 2- and the 4-gram series have separated somewhat, the former having fallen slightly while the latter continued to rise. Their ranges of standard deviation are even farther above that of the control series than they were at the end of the half hour. There should, consequently, be little confusion between animals to which these amounts of sugar had been fed and fasting animals. In the same way there should be little confusion between the 1- and the 4-gram series. On the other hand it would not be possible to certainly distinguish individuals of the 2-gram series from either of the others to which sugar had been fed.

*4.* At the end of 2 hours the mean for each of the three experimental series has fallen markedly; that for the 1-gram series being even below that for the controls. This taken together with the position of the range indicates that while under these conditions there may be a

slight hypoglycemia it is so slight that it would not be revealed in isolated observations and the series may be considered as having returned to normal. The means for the other two series have fallen even more rapidly than did that for the 1-gram series, and of the two that for the 4-gram series has fallen the most rapidly. Overlapping has increased among all of the series, and consequently the possibility of distinguishing between the series in isolated observations is becoming very small.

*5.* After 3 hours the mean for the 1-gram series has fallen far below that of the controls, and so much of the range of standard deviation now lies below that of the controls that one might hope to distinguish about 80 per cent of such animals from the controls at this time by a *hypoglycemia*. The other two series are apparently approaching a similar condition.

DISCUSSION AND INTERPRETATION. Evidently in alimentary glycemia two factors are involved. These are: *1*, the *absorption* of glucose from the intestine, and *2*, its *elimination* from the blood stream. It is also evident that the condition of glycemia at any given moment depends upon the relative rates at which these two processes are taking place. If the absorption is the more rapid, there will be an increasing glycemia, while the reverse will be true if elimination is the more rapid, and if the two are going on at equal rates the glycemia will be maintained at a constant level.

From the general laws of physical chemistry it is probable, other things being equal, that the rate of absorption of a given substance will increase as its concentration in the intestine rises above that of the blood. From this, a rapid initial rate of absorption would drive up the concentration in the blood and so the rate of elimination. There probably would come a time, however, when the rates of the two processes were equal and the glycemia would be maintained for a longer or shorter time at a relatively high and constant level. Matters do not seem to be quite so simple as this, however, for while, as would be expected from the above, the level after half an hour is higher when 2 grams have been fed than it is after 1 gram, there is no difference in the level assumed at this time by the 2- and the 4-gram series. It is hard to see why the rates of removal from the blood should not be the same in these two series, for the degree of glycemia was found to be the same and, from the plan of the experiment, the general body conditions of the animals must be assumed to be the same. On this basis there could have been no difference in the rates of absorption, and so one is driven to the conclusion that there is a definite upper limit to

the rate at which glucose can be absorbed from the intestine, regardless of the amount ingested. The mechanism of this is probably bound up with the facts revealed in Heidenhain's classical experiment, but we will not enter further into this now as it is the fact rather than the mechanism in which we are interested. Judged from the present experiments this limit, for the rabbits studied, was reached when somewhat less than 2 grams of glucose per kilogram of body weight were fed.

If the rate of absorption is no more rapid after feeding 4 grams than it is after 2 grams are fed, we should expect the maximum rate of absorption to extend over a longer period for the former than for the latter case, as there would be a larger supply in the intestine and so the intestinal concentration would be maintained above the minimum required for the maximum rate of absorption for a longer period. If, now, the rates of absorption and elimination from the blood have not come into equilibrium, after half an hour, sugar would continue to accumulate in the blood until such a time as equilibrium did occur. This is illustrated in the 4-gram series and by the "delayed maximum" found in normal men when large doses of glucose are given, and which is said by some to resemble the diabetic curve.

According to the proposed interpretation, the rate of absorption will fall, and at an increasing rate, as the intestinal concentration is reduced below the minimal amount required to maintain the maximum rate of absorption. Removal of sugar from the blood will, for a time, take place somewhat more rapidly than absorption and the glycemia will be reduced, provided the store houses have not become overloaded. In the case of diabetics, the store houses are to a greater or less degree eliminated functionally, so that elimination from the blood must take place largely or wholly through the kidneys, and so the process is delayed. This in turn delays the absorption and results in the long-maintained high level shown in the typical diabetic curve.

From the above it follows that the higher the amount of sugar fed and the consequent higher glycemia, the more rapid will be the fall after it once begins. This is illustrated by the curves shown in figure 3.

At first thought it might appear that this is not in accordance with what Strouse (8) implies when he likens the curves obtained from normal men who have received heavy doses of glucose to the diabetic curve and says that the difference between normal and diabetic men is only quantitative and not qualitative. However, it is quite possible that, had the curve for the men been studied after it had begun to fall, the rapid decline would have been as evident as it is in the case of the rabbits.

The most striking feature of the second- and third-hour periods is the very considerable hypoglycemia which is developed in the 1-gram series during this time. While this is not a new observation (9), (10), (11), it is more strikingly shown in this series than is usually the case. We offer the following as a possible explanation of this phenomenon. During the period of hyperglycemia due to rapid absorption of glucose, the store houses may be considered as having become surcharged with glycogen and glucose. This would include the muscles and other seats of active release of energy as well as the liver. Now if, as seems probable, the degree of glycemia is ordinarily determined by the requirements for glucose of the tissues in which oxidation is taking place, a low glycemia would be expected at this time because of the plentiful store already present in these tissues. Though this explanation differs considerably from the one proposed by MacLean and deWesselow it is in essential agreement with the one proposed by Folin and Berglund.

It is the usual clinical practice in alimentary glycemia tests to administer 100 grams of glucose regardless of the weight of the subject. Since adult individuals in a normal condition of flesh vary from less than 50 to nearly 100 kilograms, it results that in following this practice anything from 1 to 2 grams per kilogram of body weight may be fed. From figure 3 it will be seen that, at least for rabbits and on the basis of the above interpretations of our results, this covers a range of great variation in the rate of absorption and consequently a considerable variation in the form of the curve in individuals of different weights due to this cause alone. This variation in the curves would be confused with variations due to functional disturbance and should be eliminated if possible. This, it seems to us, could be done if a standard dosage per kilogram of body weight were substituted for the usual standard dose for an individual regardless of weight. It would further appear that the ideal for such a dose would be slightly more than enough to give the maximum rate of absorption. For the rabbit, 2 grams per kilogram would seem to be a good value to adopt.

## SUMMARY

1. It is desirable to use herbivorous as well as carnivorous animals in the study of carbohydrate metabolism. And though rabbits may not be so consistent as dogs, and perhaps men, in their blood sugar content, nevertheless much may be learned from them if their variations are properly taken into account.

2. The concentration of sugar in the arterial blood of normal fasting

rabbits, when determined by MacLean's method and presumably also by that of Folin and Wu, is 118 mgm. per 100 cc. of blood with a standard deviation of about ± 22 mgm.

3. When rabbits are fed 1 gram of glucose per kgm. of body weight, there is a sharp rise in the concentration of sugar in the blood which reaches a maximum at about ½ hour after feeding, falls to normal at the end of 2 hours, and is frequently followed by a distinct hypoglycemia 3 hours after ingestion of the glucose.

4. When 2 or 4 grams per kgm. are fed, there is a tendency to form a plateau at the maximum which is more marked and is maintained for a longer time with the larger amount and hypoglycemia is not apparent during the 3-hour period studied.

5. The rate of descent is more rapid the higher the concentration of sugar in the blood, after it has once begun, though heavy doses will delay its onset.

6. There seems to be a maximum rate of absorption from the intestine beyond which the organism can not go, regardless of the amount of sugar fed.

7. Because of the rather striking variations in the characteristics of the curves obtained after feeding different amounts of sugar, it is suggested that the standard dosage of glucose be adjusted to the body weight in sugar tolerance tests.

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# THE DIRECT INFLUENCE OF THE BLOOD OF PARATHYROID TETANY ANIMALS ON THE EXCITABILITY OF THE MOTOR NERVES

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In the study of the physiology of the parathyroid glands, proof of the existence of one or more toxins in the blood appearing coincidently with the symptoms of tetany would be significant. The effects of parathyroidectomy manifesting themselves in various parts of the body as they usually do, suggests the probability of an alteration in the circulating blood, the removal of some inhibitory substance normally present, or the abnormal presence of toxins. The experiments cited by MacCallum[1] in which blood was transfused in an isolated extremity and the electrical excitability of nerves studied, seem to show that there is a marked difference in reaction to electrical stimulation relative to the blood transfused, depending upon whether it is from a normal animal or one in tetany.

According to MacCallum, if an extremity of an animal in tetany is isolated as to its blood supply and tranfused with blood from a normal animal, the excitability of the nerves of that extremity approximates the normal. Subsequent transfusion with tetany blood results in increased reaction to electrical stimulation. The excitability of nerves in an isolated extremity of a normal animal can likewise be increased by transfusion with blood from an animal in tetany.

Because of the significance of these results an attempt was made, at Doctor Carlson's suggestion, to repeat MacCallum's experiments. Our methods of control, however, differ somewhat from those reported by MacCallum.

*Method.* Medium-sized dogs were completely thyro-parathyroidectomized. When symptoms of tetany became marked, they were anesthetized and a crossed circulation established with a normal animal.

[1] MacCallum: Mitt. Grenzgeb. Med. u. Chir., 1913, xxv, 941; Journ. Exper. Med., 1913, xviii, 618.

The carotid artery and external jugular vein of the one were connected, by means of paraffined glass cannulas, with the femoral artery and vein of the other animal. Circulation and observations were continued as long as possible under the difficulties of the operation, coagulation not infrequently obstructing the flow. Tests of the electrical excitability were made at intervals, practically simultaneous comparisons being made of the leg transfused with that of the opposite leg having normal circulation for control.

Among the different factors to which experimental variations or errors might be attributed, the following were given special attention and, wherever possible, attempts were made to reduce the chance of error to a minimum. Slight variations in the mode of application of the electrodes seemed to make little, if any difference. However, before the experiments were begun the hair was shaved off at the points of intended stimulation and care was taken at the time of testing that the electrodes and contact areas were well moistened, and that successive tests were made with the application of the electrodes to points previously marked as giving the most satisfactory response. The other factors pertained chiefly to the operation of transfusion and the relative conditions of the animals tested. *a*, Asphyxiation of the tissues of the transfused leg during the anastomosis operation, by accidental kinking of vessels and later by coagulation might result in a variation of electrical excitability. To obviate this, in each case the operation was performed as quickly as possible and the experiment terminated as soon as coagulation in the vessels was noted. Control tests on normal animals, surgical technic being the same as in the tetany experiments, seemed to indicate that a possible asphyxia of the transfused leg produced no appreciable source of error. *b*, Variation in the depth of anesthesia between the donor and the recipient was considered but in all animals as light an anesthesia as it was possible to work with was uniformly maintained. *c*, Variation in temperature between the blood of the tetany dog and that of the normal dog is perhaps the most important factor, aside from the supposed chemical changes which could be productive of the changes in our readings. Tetany animals have a pyrexia parallel with the severity of symptoms. The question is raised whether or not the elevation in temperature of the blood of a tetany dog might be sufficient to increase the electrical excitability of the nerves of a normal dog under the conditions of these experiments. Unfortunately, temperature records of the dogs here tested were not made.

The effect of transfusion was determined by a comparison of the electrical excitability of the transfused leg with that of the leg whose circulation had not been disturbed (labelled "control leg" in the tables of results) rather than by successive changes in readings, before and after transfusion. Our conclusions were based largely on changes in the cathodal closing contractions (CCC) but anodal closing (ACC) and anodal and cathodal opening contractions (AOC and COC) were also recorded in most cases.

As indicated above, a special series of control experiments was conducted in order to determine variations under normal conditions, whether or not the operation of transfusing normal blood into the leg

TABLE 1

*Control experiment: Transfusion of normal blood into one leg of normal animal*

| | | ACC | AOC | CCC | COC |
|---|---|---|---|---|---|
| Dog I | Transfused leg | 7.0 | 6.0 | 3.0 | 17 |
| | Control leg | 7.0 | 6.0 | 3.0 | 17 |
| Dog II | Transfused leg | 4.25–6.0 | 11.0–11.5 | 2.0 | 19 |
| | Control leg | 4.75–6.5 | 11 0–12.5 | 2.0 | 19 |
| Dog III | Transfused leg | 5.5.2 | 7.5– 6.5 | 3.25 | 7 |
| | Control leg | 5.0 –3.5 | 8.5– 8.5 | 3.25 | 15 |
| Dog IV | Transfused leg | 7.5 | | 4.5 | 14 |
| | Control leg | 9.0 | | 4.5 | 14 |

of normal dog produced any appreciable change in electrical excitability. The results are recorded in table 1.

In dogs 2 and 3 the two observations recorded in the case of ACC and AOC, respectively, represent observations taken (ACC) 5 and 20 minutes, and (AOC) 10 and 20 minutes after the crossed circulation was established. The indications are that the electrical excitability remains practically unchanged during the period of transfusion, even for 20 minutes. But it must be recognized that the electrical excitability varies not only between so-called normal animals but also from time to time in the same animal.

In five dogs in severe tetany one leg was transfused with blood from a normal dog. The results are given in table 2.

It will be seen from the table that excepting in cases of CCC observations of no. IV it required less current to cause a reaction in the extremity

TABLE 2

*Transfusion of normal blood into one leg of dogs in severe tetany*

| | | ACC | AOC | CCC | COC |
|---|---|---|---|---|---|
| Dog I | Transfused leg | 5.2 | 3.0 | 5.0 | 18.0 |
| | Control leg | 4.5 | 2.0 | 3.0 | 10.0 |
| Dog II | Transfused leg | 3.0 | 2.5 | 1.5 | 4.0 |
| | Control leg | 1.2 | 1.5 | 1.0 | 2.0 |
| Dog III | Transfused leg | 3.5 | 2.5–3.5 | 1.25 | 4.5–5.0 |
| | Control leg | 2.0–2.5 | 1.75 | 0.75–1.0 | 3.5 |
| Dog IV | Transfused leg | 2.5 | 4.0 | 4.5 | 8.00 |
| | Control leg | 1.75 | 3.0 | 4.75 | 7.50 |
| Dog V | Transfused leg | 4.0 | 4.0 | 2.5 | 20.0 |
| | Control leg | 2.5 | 2.5 | 1.0 | 15.0 |

receiving blood from its own body in tetany than from the normal dog.

In five normal dogs, one leg was transfused with blood from an animal in severe tetany (different tetany animals were used for each experiment). The results are recorded in table 3.

We note again considerable variation between different normal animals, but on the whole this group required stronger current than the

TABLE 3

*Transfusion of tetany blood into one leg of normal dog*

| | | ACC | AOC | CCC | COC |
|---|---|---|---|---|---|
| Dog I | Transfused leg | 8.5 | 15.0 | 8.0 | 10.0 |
| | Control leg | 10.0 | 20.0 | 8.0 | 10.0 |
| Dog II | Transfused leg | 7.5 | 5.5 to 6.5 | 4.5 | 14.0 |
| | Control leg | 7.5 | 7.5 | 6.0 | 20.0 |
| Dog III | Transfused leg | 10.0 | 7.0 | 5.5 | 8.0 |
| | Control leg | 10.0 | 14.0 | 7.5 | 20.0 |
| Dog IV | Transfused leg | 5.5 | 4.5 | 2.5 | 7.5 |
| | Control leg | 7.5 | 16.0 | 3.5 | 20.0 |
| Dog V | Transfused leg | 4.5 | 10.0 | 3.0 | 20.0 |
| | Control leg | 6.0 | 17.0 | 4.53 | 20.0 |

tetany group (table 2). Transfusion of tetany blood into one leg of a normal animal causes in the majority of the experiments a demonstrable increase in excitability of the nerves in that leg.

While the results here recorded are not as conclusive as those reported by MacCallum, they may be interpreted as supporting the theory of a chemical change in the tetany blood directly affecting nerve excitability. But before MacCallum's and our own data can be accepted as proving this point, it must be shown that the results are not due to temperature change in the transfused leg.

# THE OCCURRENCE OF CITRIC ACID IN SWEAT

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The presence of a substance yielding pentabromacetone and giving the Denigès reaction in quantitative amounts, and thus indicating appreciable quantities of citric acid, was first demonstrated in human urine by Amberg and McClure (1). By painstaking purification this substance has been more completely identified as citric acid by Amberg and Maver (2). It became of interest to determine whether or not this substance was also a normal constituent of the sweat, which eliminates so many other waste compounds that are contained in urine. Accordingly, it was at the suggestion of Doctor Amberg and Miss Maver, and under their direction, that this work was undertaken.

*Experimental.* Two types of sweat were collected, heat and work sweat. The heat sweat was collected in the steam room of a Turkish bath, with the temperature at 45°C. The subject stood naked in a large white enamel photographer's developing pan, previously cleaned and dried. At the close of the collecting period, the body was sponged off, and the sponge and pan were rinsed with a small known amount of distilled water. The subject was the same in the first three cases,—in normal health, and weighing 161 pounds. The fourth was a composite sample from four subjects, all in normal health, and with weights varying from 142 to 161 pounds. Preserved with a few drops of toluol, the sweat was carried to the laboratory in well stoppered bottles, and its evaporation was begun without delay. It was evaporated on a water bath almost to dryness, and then taken up with definite amounts of distilled water. Determinations were made in duplicate and, where the quantity collected was sufficient, by both the pentabromacetone method of Kunz (3) as modified by Amberg and McClure (1), and the Denigès reaction method of Salant and Wise (4).

The work sweat was all collected from the same subject, in normal health, and weighing 161 pounds. A suit of heavy woolen underwear

and a pair of woolen socks were boiled in distilled water for 2 hours (qualitative tests on which were blank), dried, and worn during the period of moderate and intermittent exercise on temperate days during May and June. The exercise was tennis, bicycle riding and running. At the conclusion of the period of exercise, the suit and socks were boiled for 2 hours in 5 liters of distilled water, and thoroughly wrung out by roller wringers at as high pressure as could be turned. This boiling and wringing of the woolens were at once repeated, in order to insure complete extraction, and the two samples were combined for the final determination. The collected fluids were evaporated almost to dryness over water baths, and determinations in duplicate were made by both the pentabromacetone method and the method of Salant and Wise.

TABLE 1

*Amount of citric acid obtained from human heat sweat*

| DURATION OF SWEATING PERIOD | AMOUNT OF SWEAT | pH | CITRIC ACID PLUS 1 $H_2O$ | | | |
|---|---|---|---|---|---|---|
| | | | For amount collected | | For 24 hours (calculated) | |
| | | | Salant and Wise method | Pentabrom-acetone method | Salant and Wise method | Pentabrom-acetone method |
| *minutes* | *cc.* | | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| 50 | 230 | 6.7 | 2.7 | | 77.7 | |
| 60 | 540 | 6.5 | 3.1 | | 74.4 | |
| 100 | 910 | 6.9 | 4.9 | | 70.5 | |
| 280 | 2730 | 6.6 | 13.5 | 17.3 | 68.8 | 88.2 |

For human heat sweat on a 24-hour basis, fairly constant amounts of citric acid were found for the four samples examined, as shown in table 1. These quantities varied from 68.8 to 77.7 mgm. of citric acid plus one molecule of water calculated for a 24-hour sample. In the first three cases only enough sweat was available for citric acid determination by one method, according to qualitative tests made on sweat samples previously obtained. In these cases the method of Salant and Wise was used, since the pentabromacetone method is suitable only for material from which at least more than 10 mgm. of pentabromacetone precipitate can be secured, as the final citric acid figures by the pentabromacetone method include a constant addendum of 5.5 mgm. which takes account of an undeterminable fraction found to exist by Amberg and McClure (1).

The amount of citric acid per 24 hours of human work sweat was not

as high on the average as for human heat sweat. As shown in table 2, it ranged from 54.7 to 65.2 mgm. per 24 hours by the method of Salant and Wise, and from 53.7 to 82.2 mgm. per 24 hours by the pentabromacetone method.

Hydrion determinations by a colorimetric method were made on some of the samples, as noted in table 1, by Dr. A. R. C. Haas. These showed both heat and work sweat to be acid in reaction, as recently noted by Talbert (5), although the figures here presented do not reveal as high a degree of acidity as Talbert's. Dr. E. B. Fred was kind enough to make a bacteriological examination of some of the samples, which demonstrated the presence of a single organism resembling the colon bacillus.

TABLE 2

*Amount of citric acid obtained from human work sweat*

| DURATION OF SWEATING PERIOD | CITRIC ACID PLUS 1 $H_2O$ | | | |
|---|---|---|---|---|
| | For amount collected | | For 24 hours (calculated) | |
| | Salant and Wise method | Pentabrom-acetone method | Salant and Wise method | Pentabrom-acetone method |
| *minutes* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| 150 | 6.6 | | 63.3 | |
| 210 | 8.8 | 12.1 | 59.8 | 82.2 |
| 300 | 13.6 | 11.1 | 65.2 | 53.7 |
| 420 | 16.1 | 22.8 | 54.7 | 76.5 |

In connection with this work, large quantities of saliva were collected from the various laboratory workers, preserved by a few drops of toluol until enough had been collected to work upon, and were then examined by both the Salant and Wise and the pentabromacetone methods for the presence of citric acid. The results were absolutely negative.

Discussion. No relation seems to exist between the amount of sweat secreted and its citric acid content, but the citric acid is roughly proportional to the sweating period, and thus for a 24-hour period is fairly constant. However, the figure varies for the type of sweat collected, and for the method of determination, and moreover, there is little agreement between the two methods. This is in general accord with the recent report by McClure and Sauer (6) on the citric content of urine as determined by the two methods. McClure, by the way, has devised an improved technique for the quantitative determination of

citric acid in urine by the pentabromacetone method (7), which was unavailable at the time the work here reported was done.

The collection of the heat sweat was far more fatiguing and distressing than the collection of the work sweat. When the first sample of heat sweat was secured on subject L., it took 14 minutes for the visible sweat to appear. With each subsequent period of heat sweating for this subject, the latent period before the appearance of visible sweat shortened by several minutes, although the sweating periods were a week apart. The heat sweating invariably made the subjects very hungry. One felt that peculiar "gone" feeling in the abdominal region, which perhaps best describes this hunger sensation, and this was by no means normal, for in every case the sweat collection was made not later than 2 hours after a hearty breakfast or luncheon. The sensation of hunger was accompanied by a feeling of general weakness and a slight dizziness. It is probable that these sensations were caused not so much by the actual sweating as by the profound blood changes induced by the high temperature. Dr. A. E. Koehler and myself found these same symptoms after a half-hour's immersion in a water bath at a temperature of 45°C., and in each case, in addition to the marked cutaneous vasodilatation, we found the blood to be very alkaline, and low in carbon dioxide. Under the conditions of the bath, there was no sweating in these cases comparable to that induced by standing naked in the steam room of a Turkish bath.

It is to be doubted that work sweat differs markedly in citric acid content from heat sweat. The smaller quantity of citric acid demonstrated in work sweat per 24 hours may have been due simply to the differences in the method of collection. The amount of sweating in the work performed, for the time period, and under the conditions, was not in excess of the quantity of sweat which would be secreted by an able-bodied laborer during the same time period, and doing his customary daily labor.

The figures obtained for citric acid excretion in sweat, even under the abnormal conditions of heat and work, do not approach for a 24-hour period, the amount excreted per day in the urine as reported by Amberg and McClure (1), and by McClure and Sauer (6). In normal conditions, without excess of heat or work, where visible sweat seldom appears, the amount of citric acid excreted per day in the sweat must be much smaller than the figures here reported indicate. Yet it may function both in rendering the normal sweat acid, and in maintaining the optimum conditions for the preservation of the normal acid-base equilibrium of the body.

SUMMARY

Determinations by both the pentabromacetone method and by the method of Salant and Wise show the presence in human sweat of a substance we are justified in believing to be citric acid.

For heat sweat there are 72.8 mgm. of citric acid on the average excreted per day, as determined by the method of Salant and Wise; for work sweat on the same basis, 60.7 mgm. by the method of Salant and Wise, and 70.8 mgm. by the pentabromacetone method.

For individual samples, the two methods do not give results in good agreement.

Human heat sweat is acid in reaction, individual samples having an average pH of 6.67.

Human saliva does not contain citric acid.

I wish to thank Doctor Amberg and Miss Maver for their unfailing interest and encouragement in this work, and Doctor Haas and Doctor Fred for their kindnesses in making determinations.

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# PROOF THAT A HIGH PROTEIN DIET INCREASES AND STARVATION DECREASES THE CATALASE CONTENT OF THE ENTIRE ANIMAL

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In a previous paper we (1) found that the ingestion of a single large meal of meat increased the blood catalase parallel with the increase produced in metabolism and that the ingestion of large amounts of meat for several successive days raised the blood catalase to a higher level corresponding with the rise in basal metabolism to a higher level.

The object of the present investigation was to determine if a high protein diet increases only the blood catalase or whether it increases the catalase content of the tissues and hence of the whole animal. Since the catalase of the blood is found only in the formed elements, particularly the red blood cells, the rise in the blood catalase might be due simply to a concentration of the blood. Such an increase of course would be of no particular significance so far as metabolism is concerned.

The animals used in the experiments were ordinary adult white mice. These were killed, skinned and the entire body ground through a small hashing machine three times. Precautions were taken to mix the material thoroughly. Catalase determinations were made by adding 1 gram of the ground material to 150 cc. of neutral hydrogen peroxide and the amount of oxygen liberated in 10 minutes was taken as a measure of the catalase content of the gram of material.

One hundred mice were put in a cage and fed a mixture of oats, hemp, sunflower and buckwheat weeds, the normal diet. Sixty mice were placed in another cage and fed cheese. Sixty other mice were fed bacon and boiled Irish potatoes, and 75 others were permitted to have only water. Twenty of the mice which had been kept for several weeks on a mixture of the seeds were removed from the cage, killed, ground up and catalase determinations made of 1 gram of the ground material of each mouse. The average amount of oxygen liberated by one gram of these mice was 506 cc. as shown in figure 1. After 5, 10, 15 and 20 days determinations similar to the preceding were made

using the same number of mice for each determination. The results are given on the curve marked *mixed diet.* It may be seen that the average amount of oxygen liberated by 1 gram of the ground mice was 497, 520, 510 and 530 cc. of oxygen respectively.

Twenty of the mice were removed from the cage where they had been fed cheese for 15 days, killed, skinned and ground up separately. On the curve marked *high protein diet* it may be seen that the average amount of oxygen liberated by 1 gram of the ground material in 10 minutes from 150 cc. of neutral hydrogen peroxide was 783 cc. The remaining mice in the cage were given a mixture of the seeds instead of the cheese and 5 days later determinations similar to the preceding were made using 20 mice and it may be seen that the average amount of oxygen liberated by 1 gram of the ground mice was 511 cc.

On the curve marked *low protein diet* it may be seen that the average amount of oxygen liberated by the mice which had been fed fat bacon and boiled potatoes for 15 days was 455 cc. The remaining mice in this cage were given a mixture of the seeds and after 5 days it may be seen that the average amount of oxygen liberated by 1 gram of the ground material was 545 cc.

Similar determinations were made using 20 mice which had been kept for 5 days on water. It may be seen on the curve marked *starvation* that the average amount of oxygen liberated by these mice was 353 cc. The remaining mice which had been kept on water for 5 days were then fed a mixture of the seeds for 15 days and it may be seen that the average amount of oxygen liberated by these mice was 485 cc.

By comparing the curves in figure 1 it will be seen that the high protein diet greatly increased the catalase content of the mice, low protein diet decreased catalase slightly, starvation decreased it greatly, in keeping with the fact that a high protein diet produces a great increase in metabolism while starvation greatly decreases metabolism.

Lusk (2) found that the ingestion of amino acids increased metabolism and hence attributed the specific dynamic action of meat to the amino acids resulting from the digestion of this material. The object of the experiments to be described now was to determine if the ingestion of an amino acid such as glycocoll would produce an increase in the blood catalase of rabbits.

Five grams per kilo of body weight were given in saturated solution by means of a stomach tube. Catalase determinations were made by adding 1 cc. of the rabbit's blood, diluted 1:3 with 0.9 per cent sodium chloride, to 150 cc. neutral hydrogen peroxide, and the amount of

oxygen liberated in 10 minutes was taken as a measure of the catalase content of the blood. The results of the determinations are shown in figure 2.

It may be seen that the blood of rabbit 1 while on basal metabolism liberated 1260 cc. of oxygen and that an hour after the introduction of 5 grams of glycocoll per kilo of body weight into the stomach a similar amount of blood liberated 1460 cc. of oxygen in 10 minutes.

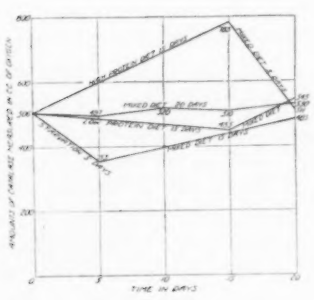


Fig. 1

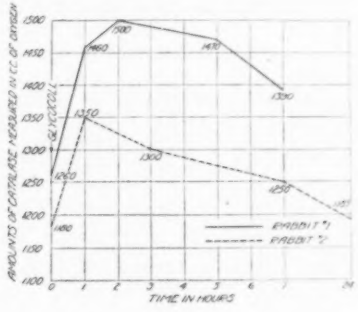


Fig. 2

Fig. 1. Curves showing the effect of a high protein diet, mixed diet, low protein diet and starvation on the catalase content of mice.

Fig. 2. Curves showing the increase in the blood catalase of rabbits produced by the introducton of 5 grams of glycocoll per kilo of body weight into the stomach.

After 2 hours the blood liberated 1500 cc. of oxygen; after 5 hours, 1470, and after 7 hours, 1390 cc. of oxygen. It may also be seen that similar results were obtained with rabbit 2 and that after 24 hours the catalase content of the blood of this rabbit had returned to normal.

## SUMMARY

A high protein diet produces an increase while low protein and starvation produces a decrease in the catalase content of the entire animal, in keeping with the fact that a high protein diet increases metabolism while low protein and starvation decrease it.

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# THE LIVING PERITONEUM AS A DIALYZING MEMBRANE

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The laws regulating the absorption of solutions injected into the peritoneal cavity have been the subject of many researches. They are of the greatest practical importance in medicine and surgery, and also of great theoretical interest to physiology, bearing, as they do, upon the problems of lymph production, the activity of cells in general, and of the serosa cells in particular, and upon the osmotic properties of the blood. But the passage of dissolved substances in the opposite direction—from the blood-stream into fluids in the peritoneum—has received only passing attention, although its theoretical interest is also great, and there exists the possibility of its practical application. It is the purpose of this study to investigate and record the degree and speed of the diffusion of various solutes from the blood through the peritoneal membrane, to review the physiology of the peritoneum in the light of any new facts thus obtained, and to canvass the possibility of the application of the method to other physiological problems.

The experiments bearing on the question in hand which have been reported by previous investigators, have mostly been incidental to researches on the laws of absorption into the blood-stream. Wegner (1) found that injection of hypertonic solutions of sugar, salt or glycerine caused an exudation in the dog's peritoneal cavity, which might reach 8 per cent of the body weight in 23 hours. But apparently he did not analyze his exudates. Starling and Tubby (2) injected distilled water and solutions of sodium chloride and of sugar, of various strengths, into the pleural cavities of dogs, and found that hypertonic solutions increased in volume temporarily, but decreased in concentration, and proteins and salt appeared in them.

Perhaps the most complete study of the relation between the respective concentrations of solutes in the blood and in fluid in the peritoneal cavity is that of Orlow (3), a pupil of Heidenhain. Orlow gives the protocols of a long series of careful experiments in which he injected

sera and salt solutions of various strengths into the peritoneal sacs of dogs, analyzing the injected fluid, blood, serum, and sometimes thoracic duct lymph before and after. An increase in salt content occurred in solutions originally containing 0.3 per cent sodium chloride (freezing point about −0.18), although they decreased in volume. Salt solutions of a concentration of about 1.5 per cent (freezing point about −0.88) or more caused a marked transudation of fluid from the blood stream, but a rise in blood chlorides. Solutions containing from 0.4 per cent to 1.5 per cent of salt diminished in volume (during the interval allowed —usually about 3 hours) and tended to approach the chloride concentration of the blood-serum. Injection of hypertonic salt solution intravenously hastened the absorption of peritoneal fluid and the diffusion of salt from the blood-stream. But the changes in fluid volume and concentration were not in accordance with simple osmotic laws (the freezing point of dog's blood being −0.571 (4)). Orlow states that the presence of 0.05 per cent to 0.08 per cent sodium fluoride in salt solutions of 0.4 per cent to 0.6 per cent caused them to increase in volume in the peritoneal cavity; but he gives no exact experimental data to prove the point. As a result of his comprehensive and painstaking study, he contents himself moderately with Heidenhain's conclusion that absorption from the peritoneal cavity is a vital process.

Orlow observed that the injection of hypertonic salt solutions intravenously increased the speed of absorption of peritoneal fluids. Fleisher and Loeb (5) found the absorption of fluid to be increased in animals without kidneys or with obstructed ureters, which they attributed to a rise in the osmotic pressure of the blood.

Other substances in solution besides sodium chlodide have been used to produce peritoneal exudates. Of these the most important is glycerine, which has been used by Wegner (1), Schnitzler and Ewald (6), Hertzler (7), Schrader (8) and others. In Schrader's series of experiments, which seems to be the most extensive and complete one, small quantities of 90 per cent glycerine (the concentration usually available) were injected into the peritoneal cavities of rabbits. Such solutions should have a freezing point of about −21. Amounts up to 10 cc. were survived, and gave rise to effusions of 50 to 90 cc. of clear or slightly bloody fluid, containing 0.2 to 0.3 cc. of glycerine. No further analyses of the resulting fluids are given. At autopsy, the peritoneum was rarely found to be grossly injected. Glycerine does not cause any exudation when injected subcutaneously (6). Hertzler found that formaldehyde was the only other drug, out of a number

which he tried, which delayed the absorption of fluids. The injection of a 2 per cent solution caused an increase of 50 per cent in volume in 2 hours, and the fluid was not absorbed for 12 hours or longer. Hertzler attributes the delay in absorption to the presence of albumen in the transudate. It will be seen, however, that in the case of glycerine at least, the fluid is strongly hypertonic.

*Methods employed in the present study.* Cats were used as experimental animals for the most part, but observations were also made upon a few rabbits and dogs. Aside from the size of the animals, and the amount of fluid employed, there appeared to be no essential difference between the three species as far as the conclusions to be drawn from these experiments are concerned. Ether was given by cone during all procedures causing pain, except in the cases where other anesthetics are specified. The animals were allowed to survive the experiments whenever possible, which they usually did without apparent distress or ill-health, and some of them were used for three and four successive perfusions. Only two or three out of the entire series became infected, although the most meager aseptic precautions were observed.

The various fluids used were introduced into the peritoneal cavity and removed through a blunt cannula, introduced into the animal's flank by means of a trocar. The trocar was accidentally inserted through the wall of a distended stomach in two instances; but in one of these the animal was allowed to survive, and was apparently little damaged. Care had to be taken not to allow the point of the cannula to remain outside of the peritoneum. At autopsy a slight ecchymosis at the place of contact with various viscera was often observed. The warmed fluid was allowed to run in at a pressure of about 20 cm. of water, or injected with a large syringe. The puncture wounds sometimes leaked a trifle during and after the operation, but healed well. A cat's peritoneal sac will hold a volume of fluid equivalent to about one-tenth of the body weight, apparently without causing discomfort. In one series of experiments fluid was allowed to flow in and out of the peritoneal cavity continuously, usually at the rate of about 500 cc. in 2 hours. The outflow cannula frequently became plugged by the impingement of viscera or of the omentum. This difficulty was overcome by the use of a sterile silver probe fitting loosely in the tube with which the obstruction could be pushed away from time to time, although occasionally only at the expense of slight bleeding.

Blood for analysis was drawn by subcutaneous puncture of the femoral artery, or from the heart after death. The saphenous vein was used for injections.

Urea in the blood and fluid was determined by Marshall's urease method, with aeration of the ammonia by suction, and titration with N/100 acid and alkali. The chlorides were determined by picric acid and silver nitrate precipitation, and titration with potassium iodide as suggested by Van Slyke and Donleavy (9). Whole blood was used for this determination, instead of plasma as usual, owing to the small amounts available for analysis. Sugar was determined by Benedict's picrate method, the final solution being compared with a standard picramic acid solution in a Hellige colorimeter. An Esbach albuminometer was used to estimate the amount of protein in the fluids obtained. Other analytical methods will be described with the experiments in which they were used.

*The osmotic equilibrium between the blood plasma and fluids in the peritoneal cavity.* The dialyzing fluids which will be considered in the present study may be divided into two general classes: those which progressively diminish in volume from the time of injection, and those which show a temporary increase in volume. According to Orlow's work (3), the dividing line between these two classes for simple salt solutions comes at a concentration of about 1.5 per cent, and this is in agreement with the results of the experiments here reported. Of course, even the fluids of the second class are eventually absorbed by an intact animal. A further division into subclasses is less satisfactory. Orlow suggests a distinction between salt solutions from which the salt is absorbed as well as water and those in which the salt content increases, the dividing line coming at a concentration of about 0.4 per cent of sodium chloride. Such a distinction seems somewhat artificial, if we regard some other solute than the one originally present in the fluid; for, as will be shown, such crystalloids as urea and sugar appear in regular and progressive quantities in fluids of various kinds in the peritoneal cavity.

Among the solutions which undergo a temporary increase in volume in the peritoneal cavity, we must perhaps recognize a class which is primarily irritating, rather than purely hypertonic. Such a solution would be that of formaldehyde, used by Hertzler (6). But many irritants do not cause a transudate of any volume (heat, mechanical irritants, croton oil, iodine in the present series, and several others tried by Hertzler). Whether the hypertonicity of salt, sugar and glycer-

ine solutions is in itself irritating, is perhaps partly a question of definition.

The presence of colloids in the peritoneal fluids doubtless delays their absorption, as was pointed out by Hertzler, and probably also the attainment of osmotic equilibrium, as will be seen in the case of dextrose solutions in the present experiments.

*Solutions progressively absorbed from the peritoneal cavity.* The injection of salt solutions of a concentration of less than 1 per cent into the peritoneal cavities of experimental animals is well borne if the solution is at body temperature and no excess is used. Such solutions are hypotonic compared with cat's blood, which, according to Mathews (4) has a freezing-point of $-0.638$ (corresponding to a 1.09 per cent sodium chloride solution). If the animals are allowed to recover from the anesthetic, they usually run about and eat and drink much as do normal animals, except for the disability of a distended abdomen. Some of the animals appear at first to be in some distress, crouch in one position, whining if disturbed, and frequently vomiting. But this distress seldom lasts more than an hour or two. The animals pass much urine during the second and third hours of the experiment but seldom defecate.

If the animal's abdomen is tapped within a few minutes after the injection, the fluid will be found already to have undergone a change. It has decreased in volume, and protein, urea, sugar and other materials have appeared in it, while the percentage of chloride has begun to approach that of the blood-plasma. Blood-cells do not appear, unless there has been a gross wound of the peritoneum, but a few large mononuclear cells may be found in it microscopically. Absorption is most rapid with distilled water, and the less concentrated salt solutions, but in comparison with the individual variation in the rate of absorption in different animals, the differences in the absorption of different solutions in this group is slight. How rapid the absorption may be is illustrated in the following protocols:

*Experiment 65.* November 8, 1921: Under deep ether anesthesia, 100 cc. of a 0.5 per cent salt solution at a temperature of 55°C. were injected into the peritoneal cavity of a small cat by means of a large syringe and blunt cannula. The injection was not completed before the animal began to breathe irregularly, and the heart stopped beating a few seconds later. At autopsy (about 3 minutes after the completion of the injection) 22 cc. of fluid containing 0.25 per cent albumin (Esbach) were recovered.

*Experiment 12.* June 6, 1921: Thirty cubic centimeters of warm sterile tap water were introduced into the peritoneal cavity of a cat by means of a cannula

and gravity bottle, under ether. There were intense fibrillary contractions of the abdominal muscles, and the heart stopped beating immediately. Ten cubic centimeters of fluid were recovered.

Usually, however, 60 to 70 per cent of the fluid injected may be recovered within 2 or 3 hours. Absorption is complete within 8 or 9 hours. Anesthesia appears in some instances to delay the rate of absorption and also of diffusion.

Before the fluid is completely absorbed, a more or less complete osmotic equilibrium with the blood plasma is reached, as may be illustrated by the following typical protocols:

*Experiment 67.* November 9, 1921: A large cat was given 50 mgm. morphin sulphate and 100 mgm. magnesium sulphate subcutaneously, and 0.6 gram chlorbutanol (chloretone) in alcohol by stomach tube. Anesthesia was rather light and required to be supplemented by ether for the operative procedures.

One hundred and fifty cubic centimeters 1 per cent warm sodium chloride solution containing 0.01 per cent trypan blue were injected into the peritoneal cavity with a syringe and needle at 10:30 a.m. When released the animal lay quiet on the floor, breathing regularly.

At 2:30 p.m. a large trocar was introduced into the right flank and the abdomen gently massaged to remove the fluid as completely as possible. Meanwhile, 10 cc. of blood were withdrawn from the femoral artery by subcutaneous puncture. At 2:45, 78 cc. of fluid were withdrawn; 10 cc. of this were reserved for analysis, and the rest warmed and reinjected.

The fluid removed contained 0.0802 per cent urea, 0.835 per cent chlorides (as NaCl), 0.225 per cent sugar. The trypan blue was determined colorimetrically against the original solution as a standard; it was diluted to 72 per cent of its original strength. The blood urea was 0.0843 per cent, chlorides 0.7 per cent, sugar 0.154 per cent (analyses all made on whole blood).

From 5:20 to 5:40 p.m. the peritoneal cavity was again drained, 18 cc. of cloudy fluid being recovered; 7 cc. of this were taken for analysis, and the rest, with 10 cc. from fresh stock to compensate for the loss, were reinjected. A sample of blood was taken from the femoral artery.

This second fluid contained 0.097 per cent urea, 0.822 per cent chloride, 0.138 per cent sugar, and 82 per cent of the original concentration of trypan blue—the reading was not accurate on account of the turbidity of the solution. The blood at this time contained 0.088 per cent urea, 0.72 per cent chlorides, and 0.122 per cent sugar.

At 7:30 p.m. the animal was sacrificed. No fluid was found in the peritoneal cavity, which was slightly injected and stained with trypan blue. A small ecchymosis was found near the point of contact of the cannula. No other lesions were observed.

*Experiment 75.* November 21, 1921: Two hundred cubic centimeters warm distilled water containing 0.01 per cent trypan blue and saturated with chlorbutanol was injected into the peritoneal cavity of a large cat under ether, at 3:20 p.m. Ether was then discontinued and was not needed for further manipu-

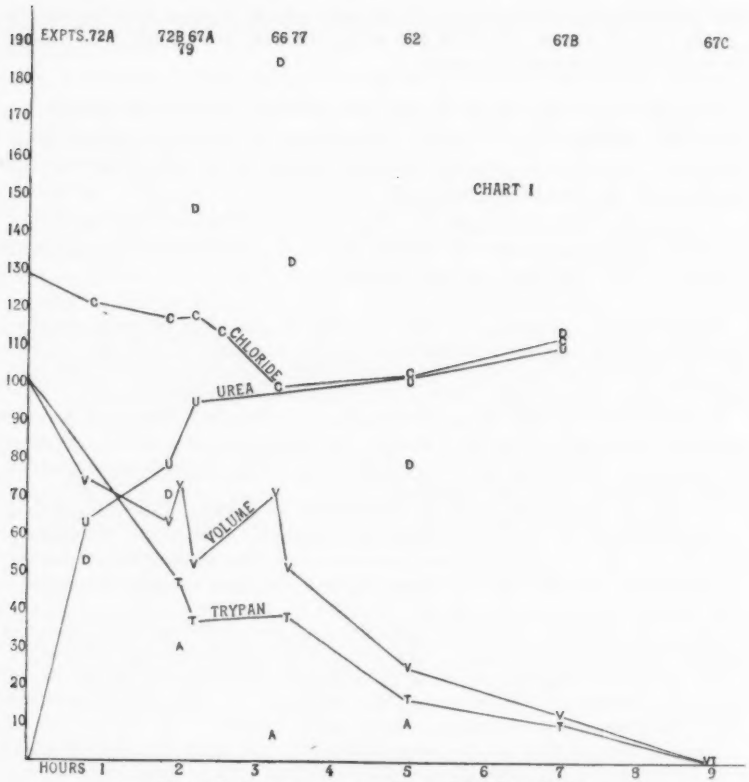


Chart I. One per cent sodium chloride solution.

*Explanation of charts.* Chart I represents a series of experiments in which 1 per cent sodium chloride solution was used as a dialyzing fluid, chart II a series in which distilled water was used. The abscissae represent the time in hours elapsing after the first injection of fluid. The experiment numbers are given at the top of the chart. The curve marked *Volume* indicates the percentage of the original amount of fluid which was recovered at various intervals. The *Trypan Blue* curve indicates the percentage of the original amount of dye injected. The *Urea* and *Chloride* curves represent the proportional percentage of these substances found in different experiments; the corresponding values for dextrose are not plotted, but are marked *D*. (For method of calculation, see text.) In chart I, the value for blood chlorides at the beginning of the experiments is taken as 0.78 per cent, the average of all the results in the series. The points marked *A* represent decigrams of protein per liter of dialysate in different experiments.

lations. At 5:30, 70 cc. of blood-tinged fluid were removed through a large trocar, the abdomen being thoroughly massaged. A sample of blood was taken from the femoral artery. The animal was then released.

The fluid contained 0.0895 per cent urea, 0.645 per cent chlorides, 0.30 per cent sugar, and 0.6 per cent albumin. The trypan blue could not be compared colorimetrically owing to contamination with blood, but the fluid appeared darker than the original solution. The blood contained 0.105 per cent urea, 0.788 per cent chlorides, and 0.22 per cent sugar.

The animal was found dead the second morning following. The lungs were injected and the bronchi contained a purulent exudate. The peritoneum was somewhat injected also.

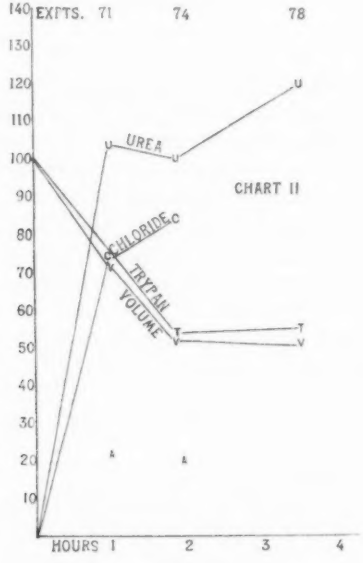


Chart II. Distilled water.

Two charts are appended, showing the progress toward equilibrium after various intervals in all of the comparable experiments in this series. The experiment numbers are given at the bottom of the chart, and may be compared with the protocols cited in the text, to make the method of calculation clearer. Owing to differences in the size of animal, volume of fluid used, and composition of the blood in different experiments, it has been necessary to reduce all the values represented to percentages in order to make the various data comparable. The

quantities plotted for the volume of the fluid and the concentration of trypan blue represent the percentage of the original volume injected and of the original concentration used, respectively, which were found at the end of the periods given. The values plotted for urea, chlorides and sugar, on the other hand, represent the proportions between the concentrations of the various solutes in the fluid, and their respective concentration in the blood at the same time. That is, the values were obtained by dividing the percentage of the solute in question found in the fluid, by the percentage in the blood removed at about the same time; they represent a proportion between percentages. The amount of albumen found in some of the experiments is plotted as decigrams per liter (Esbach). The number of different experiments recorded in each chart may explain in part the ruggedness of the curves, which may also be due to analytical errors and to individual variations of an unknown nature in different animals. But the compilation of a sufficiently long and full curve from a single experiment would involve many difficulties. As will be explained below, the values found for the proportional percentages of dextrose and the amount of dissolved protein probably deserve a different interpretation from that of the other percentages, and for that reason the points merely are plotted, but the curves are not drawn.

Solutions of sodium sulphate behave much as do isosmotic solutions of sodium chloride when injected intraperitoneally. The sulphate disappears more rapidly than does the chloride, and is quickly excreted in the urine. The plasma crystalloids appear in sulphate solutions as they do in other fluids.

*Fluids which temporarily increase in volume in the peritoneal cavity.* By increasing the salt content it is possible to produce fluids for intraperitoneal dialysis which completely retain their volume, or increase in amount, as Orlow's (3) tables show. The dialysate resulting from the use of such fluids is rich in albumin, and comes to an equilibrium with the blood-plasma quite as rapidly as do those produced by weaker solutions. Hypertonic solutions in any quantity are not well borne, however; the animals vomit and defecate, and lie on the floor whining if allowed to recover from the anesthetic. On this account, practically all of such experiments were performed under continuous light anesthesia, either with ether or with chlorbutanol. The animals pass large quantities of urine, beginning usually about 2 hours after the injection. They usually die within 12 hours, if concentrated solutions have been used, and removal of the fluid at or after its maximum volume does not

affect their survival. At autopsy there is found to be subcutaneous edema about the point of puncture, the heart is dilated and the lungs are very moist. There is moderate injection of the peritoneum, and the mesenteric nodes are enlarged. They can at times be saved by the administration of sterile water or very weak (0.2 per cent) salt solutions intravenously, in which case the increase in volume of the intraperitoneal fluid is aided, and equilibrium is brought about more rapidly and completely. The dialysate thus obtained is very rich in protein, though poor in cells, and often clots lightly on standing.

The following is a typical protocol:

*Experiment 63.* November 7, 1921: Medium sized cat. Ether anesthesia. One hundred cubic centimeters 5 per cent sodium chloride solution (freezing point −3.3) containing 0.05 per cent trypan blue, were injected into the peritoneal cavity at 11 a.m. Light anesthesia was continued for an hour, and the animal was then released. It lay on the floor in coma, whining and occasionally vomiting a bile-stained material.

At 4 p.m. the animal was anesthetized and tapped, but died before all the fluid had run out. Little ether was used. In all, 130 cc. of fluid were recovered, which on analysis showed 0.053 per cent urea, 1.17 per cent chlorides, 0.084 per cent sugar, 0.26 per cent albumin. The concentration of the trypan blue was about 30 per cent of the original. Blood taken from the heart contained 0.0603 per cent urea, 1.27 per cent chlorides, 0.07 per cent sugar.

Hypertonic solutions of dextrose and of glycerine were also used in the peritoneal cavities of cats. The injection of small amounts of glycerine produced the phenomena described by Schrader (8) as cited above. The diffusion of chlorides and sugar into glycerine-containing fluids was, however, rather slow. Dextrose syrups of a strength of 10 per cent (freezing point about −1.02), usually with the addition of 0.5 per cent sodium chloride (reducing the freezing point to about −1.31) were well borne, and proved very useful in the experiments to be described below, in which the attempt was made to extract as much as possible of some solute from the blood-stream by perfusion of the peritoneal cavity with large amounts of fluid. But, as in the case of glycerine, diffusion into such viscous solutions was slow. Solutions as strong as 20 per cent glucose (freezing point about −2.12) were poorly borne. They increased markedly in volume, and much albumen appeared in them.

*The diffusion of foreign solutes from the blood-stream into fluids in the peritoneal cavity.* Urea, dextrose and chlorides are the only normal constituents of the blood that have been demonstrated in peritoneal washings. A substance has been observed in peritoneal fluids which

is precipitated by picric acid and redissolves in alkalis to form a red solution, but other tests for creatinin have not been obtained. Uric acid has not been demonstrated in the washings even by Folin's delicate phosphotungstic acid reaction. A distinct odor, like that of fresh cat's urine, is always to be detected in the fluids obtained.

Observations have also been made upon the behavior of other dissolved substances in the blood-stream, especially those which are easily recognized or determined. In general, it may be said that the smaller the molecule of the substance employed, the more rapid and complete is the equilibrium between the blood-plasma and the peritoneal fluid. But in the intact animal, the peritoneal washings never acquire a very large proportion of a solute injected intravenously, apparently because of the rapid removal of the foreign substance by the kidney, by phagocytes, and in other ways. The method of continuous perfusion of the peritoneal cavity was usually employed in this series of experiments, because it permits the use of large volumes of fluid, and so increases the amount of material extracted, although the equilibrium is never so complete as when the fluid is introduced and allowed to remain.

The odor of ether can be made out in the peritoneal fluid in experiments where ether is the anesthetic used. So, too, chlorbutanol appears in the fluid, even when given by stomach. Of the various substances used intravenously, sodium iodide diffused most rapidly and completely through the peritoneum, appearing in the washings almost as early as the chlorides of the blood. Ferrocyanides, salicylates and phenolsulphonephthalein also found their way into the peritoneal fluid, approximately in the order named. Alizarin, acid fuchsin, Congo red, trypan blue and pyrrol blue, all more colloidal substances, did not appear in the peritoneal washings, even when administered intravenously in large amounts. Neither were they excreted by the kidney except very slowly.

The following protocols will illustrate the general methods employed, and some of the quantitative results.

*Experiment 11.*—July 4, 1921: A rabbit weighing 1,250 grams was etherized and fastened in the prone position. At 2:30 p.m., 1 cc. each of a standard solution of phenolsulphonephthalein and of a 10 per cent solution of sodium iodide were administered intravenously, and at the same time perfusion of the peritoneal cavity with 10 per cent sodium chloride was begun, the saline being introduced into one flank through a large trocar, and continuously drawn off through a trocar in the other flank, a blunt probe being employed to clear the outflow cannula as necessary. Sodium hydroxide was added to samples of the washings. The

first pink color was obtained 20 minutes after the injection of the dye. At 4:00 p.m. the pink color ceased to appear; 300 cc. had run in; 600 cc. had run out. The ether was discontinued, but the animal died a few minutes later. At autopsy the peritoneum was found to be slightly injected. The heart was firmly contracted in systole. The tissues appeared very dry.

The color of the alkalinized fluid was found to match that of 0.015 cc. of the standard phenolsulphonephthalein dilated to 500 cc. That is, 1.8 per cent of the dye injected made its way into the peritoneal fluid. The urine gave a very deep color when alkalinized.

The iodide radicle was demonstrated in the blood by the addition of starch paste and a few drops of nitroso-sulphuric acid. It was demonstrated in the peritoneal fluid by the same means, and quantitated by adding nitroso-sulphuric acid to aliquot portions, shaking out with measured amounts of chloroform, and comparing the purple coloration of the chloroform with that of various dilutions of the original solution similarly treated. Iodides (0.0075 gram, as sodium iodide,) were found in the fluid obtained—7.5 per cent of the original amount injected.

*Experiment 20.* July 29, 1921: A cat weighing 1,330 grams was etherized and fastened on its back at 9:45 a.m. Three cubic centimeters of a 10 per cent sodium salicylate solution were injected into the saphenous vein. At 10:15 a.m. a trocar was introduced into each flank and lavage with 1 per cent sodium chloride solution was begun. The outflow was examined for salicylates by acidifying slightly, and adding a few drops of a saturated ferric ammonium sulphate solution. A deep purple coloration was observed. At 12:15 a liter of saline solution had been run in, and 850 cc. obtained, the last washing giving about as deep a color as the first.

At 12:30 p.m. the perfusion of a 5 per cent sodium chloride solution was begun. The outflow yielded a coloration with the ferric salt about as deep as that observed in the first fluid. At 1:30, 500 cc. had been introduced and 600 cc. obtained.

The peritoneal cavity was allowed to drain for a few minutes, in order to empty it as completely as possible of the concentrated saline. Perfusion with sterile distilled water was begun at 2:15 p.m. The abdominal muscles on the side of the injection cannula twitched violently, but respiration and pulse were little affected. At 3:45 the animal's heart stopped beating, although only a small amount of anesthetic was being used; 500 cc. of water had run in, 400 cc. had run out. The autopsy showed the peritoneum to be pale, smooth and glistening, except for a few small points of ecchymosis. The heart was dilated and full of blood. The lungs were dry and pale. The other organs appeared grossly normal.

The blood was analyzed and found to contain 0.98 per cent chlorides, 0.016 per cent urea and 0.4 per cent sugar. Five cubic centimeters of urine were found in the bladder, containing a trace of salicylate.

The outflow from the first fluid (1 per cent saline) contained 1.0 per cent chlorides, 0.01 per cent urea, 0.048 per cent sugar. The coloration obtained by adding a ferric salt to the washing was compared with dilutions of the original sodium salicylate solution similarly treated, and the fluid was found to contain about 0.00237 per cent of salicylate.

The second washings (from 5 per cent saline) were also analyzed, and found to contain 2.0 per cent chlorides, 0.008 per cent urea, 0.048 per cent sugar and 0.0018 per cent salicylate. The outflow from the distilled water contained 0.495 per cent chlorides, 0.0088 per cent urea, 0.046 per cent sugar and 0.0023 per cent salicylate.

Adding the amounts of salicylate obtained, we find that approximately 0.04 gram was extracted in all, out of 0.3 gram injected.

*Experiment 42.* October 12, 1921: A small cat was etherized, and cannulae were inserted into the saphenous vein and into the peritoneal cavity through either flank. A 10 per cent solution of dextrose, containing also 0.5 per cent sodium chloride, was slowly perfused through the peritoneal cavity from 2:00 to 4:30 p.m. Meanwhile, 200 cc. 0.3 per cent NaCl were run into the saphenous vein from 2:00 to 3:45 p.m., and (as this dilute saline was perfectly borne) 300 cc. 0.2 per cent sodium chloride were infused from 3:45 to 4:30. All the solutions used were kept at body heat, and the animal was warmed with lamps. At 2:30 p.m. 1 cc. of the standard phenolsulphonephthalein solution was given intravenously. The animal was in good condition at 4:30 p.m., at which time 500 cc. of the syrup had been injected, and 610 cc. recovered. Ten cubic centimeters 10 per cent alizarin were then injected intravenously, the animal stopped breathing and could not be resuscitated. At autopsy all tissues were found to be wet and edematous. The peritoneum was not particularly injected. The heart was dilated. No color (alizarin) was seen in the few cubic centimeters of fluid remaining in the abdominal cavity. The bladder was empty. (An unknown amount of urine was lost.)

Analysis of the heart's blood showed 0.479 per cent chlorides, 0.0445 per cent urea.

The fluid was found to contain 0.470 per cent chlorides and 0.0791 per cent urea. Its specific gravity was 1.031. It contained 0.6 decigram albumen per liter (Esbach). When alkalinized, its color matched that of 0.075 cc. standard phenolsulphonephthalein solution dilated to 500 cc.

That is, about 9.5 per cent of the dye injected was removed by administering a hypertonic solution intraperitoneally and at the same time a hypotonic solution intravenously.

*Experiment 37.* August 17, 1921: Under ether, a moderate-sized cat was perfused with 10 per cent dextrose containing 0.5 per cent sodium chloride from 2:20 to 4:00 p.m. At 2:40, 0.1 gram trypan blue in 4 cc. distilled water was injected intravenously. At the end of the experiment 500 cc. fluid had been injected and 475 cc. obtained. At no time was any bluish coloration to be observed in the fluid. The cat was released in good condition and made a perfect recovery.

Discussion. In summarizing the results of this series of experiments, we may say that crystalline solutes and water tend to diffuse through the peritoneum and capillary endothelium both from and to the bloodstream in such a way that solutions in the peritoneal cavity come into more or less complete osmotic equilibrium with the blood plasma in respect to all of the crystalloids present in both; but that all fluids,

including hypertonic ones, tend to be absorbed even before they are diluted. The differences between the concentrations of solutes in the blood and in the plasma are such as may be accounted for by experimental errors, by the rate of excretion through the kidney, and perhaps by the space occupied by the corpuscles and solids in the blood, as suggested by v. Hess and McGuigan (10) for the vividiffusion of dextrose. The colloids (substances having a molecule larger than that of acid fuchsin or Congo red) which have been investigated, do not pass from the plasma into peritoneal fluids, although even particulate matter is absorbed from the serous cavities. Possible exceptions to these two generalizations are the cases of the coagulable protein formed in the peritoneal washings, and of dextrose. These will be discussed separately below, where an attempt will be made to show that the exception may be only apparent.

Wegner (1) could only imagine four mechanisms by means of which solutions might be absorbed from the peritoneal cavity. These were: diffusion or osmosis; filtration; cellular action—phagocytosis or potocytosis by mesothelial or wandering cells; and lymphatic absorption through the "lymphatic stomata" described by v. Recklinghausen and others. More recent investigations (by Sabin (11), MacCallum (12), Clark (13) and others) have thrown much doubt upon the supposed open communication between the serous cavities and the lymphatic vessels, and even if such be present, absorption through them would be a sort of filtration or sieve-action. *A priori*, then, it is difficult to visualize more than three methods of transmission either from or to the peritoneal cavity: passage of small molecules through a permeable or semi-permeable membrane (protoplasm or continuous intercellular substance); passage of small and large molecules, and perhaps particulate matter through microscopic or ultramicroscopic (but not intermolecular) spaces; and some form of "vital activity," of which the most obvious example is the phagocytosis of microscopic particles and droplets of fluid by the serosa cells.

To explain the passage of small molecules but not large ones, in both directions through the peritoneum (plus the endothelium), we are forced to predicate the existence of a membrane, not necessarily endowed with "vital" powers, microscopically continuous, but physically pervious to particles of the size of a molecule of phenolsulphonephthalein or smaller. Such a membrane might be cellular or intercellular. In addition, we must imagine some mechanism by which all fluids introduced into the peritoneum are progressively absorbed, together with

any particles even of microscopic dimensions, which they may contain. The experiments of Orlow (3) seem to show that the flow of thoracic duct lymph is not increased by this absorption, although colloidal and larger particles appear in the regional lymph nodes. It does not seem necessary to assume any "vital" activity of the serosa cells to account for the disappearance of hypertonic fluids and particulate matter, since it is conceivable that the intra-abdominal pressure might be great enough to force them through openings of appropriate size. On the other hand, the phagocytic powers of the mesothelial cells are well known.

A mechanical simile may serve to make clear the relation between the two types of absorption from the peritoneum.

Let us suppose a hollow cylinder, closed at the lower end by a dialyzing membrane, to be supported within a much larger vessel. The dialyzing cup may represent the potential peritoneal cavity, the larger vessel the body fluids, the membrane the peritoneum. Suppose the membrane to be of parchment or gold-beater's skin, the outer vessel to contain normal saline, and the cup to be filled, to a level higher than that of the fluid in the outer vessel, with hypertonic salt solution. How closely will the model imitate the course of events following the introduction of hypertonic saline into the peritoneal cavity of animals? The hypertonic solution will seep into the fluid in the larger vessel at a rate determined by the difference between the two fluid levels, and at the same time salt will diffuse outwards through the membrane, and water inwards. The column of fluid in the cylinder will rise, since the molecule of water is more diffusible than that of sodium chloride, and since the osmotic pressures at work greatly exceed the static pressure of the fluid in the cylinder. After a certain time the two solutions will reach a more or less complete osmotic equilibrium, and the fluid level in the dialyzing cylinder will have risen, although not to as high a level as if the dialyzing membrane were semipermeable. Seepage will go on at an increased rate meanwhile, and eventually the level of fluid within and without the cylinder will be the same also.

This is obviously different from what takes place in the animal body, where absorption of aqueous solutions injected into the peritoneal cavity is always rapid, while diffusion in either direction is comparatively slow. Thus, in experiment 63 (cited above) during 5 hours 100 cc. of 5 per cent sodium chloride increased only to a volume of 130 cc., while the blood became more concentrated in salt than the peritoneal fluid. A further qualitative difference is seen in the behavior of solutions of

colloids in the actual animal and in the model respectively. Trypan blue, for example, would be retained by a parchment membrane, but disappears from the animal's peritoneal cavity at a rate roughly parallel to that of the diminution in volume of the fluid as a whole (compare charts I and II).

We can, however, improve the likeness of the model to the living peritoneum, by pricking one or many tiny holes in the dialyzing membrane or the sides of the cylinder. These will greatly increase the seepage in the direction of static pressure, and will permit the passage of colloidal material, although diffusion will go on to some extent as before, and hypertonic solutions in the cylinder will reach a certain maximum of volume before beginning to decrease to zero. By varying the dimensions of the membrane and of the holes made in it respectively, it would be possible to imitate the course of any of the experiments in the present series.

It would be of interest in this connection to compare the proportion of various substances in the blood and transudates of ascitic patients, or in a portion of an intraperitoneal infusion, so frequently used now-a-days in pediatrics. A more physiological dialysate would be the pericardial fluid from selected human autopsies, which is normally present in quantities sufficient for analysis. The transmission of poisons, toxins and antitoxins by the peritoneum might be worth determining also. The details of a rather unsuccessful attempt to influence the course of experimental uremia will be given in a later paper (14).

*The protein in the peritoneal washings.* The only colloid appearing *de novo* in the fluids obtained by peritoneal lavage is one giving the ordinary reactions of a protein. Leaving out of account the cases in which blood-cells or fibrin flakes appeared (in which gross wounds of the peritoneum were always to be observed), few microscopic formed elements regularly accompanied the protein, which was always present in varying amounts from 0.1 per cent to 0.6 per cent. The proportion was more or less constant for each fluid used for perfusion; it was least with approximately isotonic salt solutions, and increased slightly with hypotonic solutions, markedly with the various hypertonic ones used. The possible sources of such a protein are: the normal proteinous lubricating fluid of the peritoneum (perhaps increased in amount under the conditions of the experiment); an extract of the mesothelial cells and underlying tissues; and a diffusate or exudate from the capillaries. Certainly the normal secretion of the serosa cells must be included in the washings, but if it were the only source of protein in

them, it is hard to see why the proportion should vary so much, and should never drop below a certain figure, even when the total volume of fluid used is relatively enormous. The source of the remainder is not clear from the present study alone. Against the immediate origin of the protein from the blood-stream is its small amount, its relative constancy, its lack of fibrinogen as a rule, and the failure of non-proteinous colloids to pass from the plasma into the peritoneal fluid.

*The behavior of dextrose.* In most of the experiments of this series, little or no attempt was made to control the dextrose content of the blood. The animals were not always starved before being used, ether by cone was employed in many cases, and the animals were usually wild and frightened when brought into the operating room. Such reasons as these doubtless account for the high values obtained for blood-sugar in most of the analyses, and probably large variations occurred during the course of the experiments. It is well known how sudden and extreme the fluctuations of the blood-sugar level may be. In a few experiments (cf. expers. 20, 63, 67 and 75 cited above) in which some care was taken to keep conditions uniform throughout the experiment, the fluid-dextrose content exceeded the blood-dextrose content. But the results show no such beautiful regularity as those obtained by v. Hess and McGuigan (10) by the vividiffusion method, and no conclusions of importance are to be drawn from them. It is probable that some refinement of the present method, applied to normal or to artificially diabetic animals, might be made to yield data of value.

## SUMMARY

1. The details of a simple method are given by which the flowing blood may be repeatedly dialyzed against indifferent fluids in the intact animal, using the living peritoneum as a dialyzing membrane.

2. The dialysates thus obtained vary somewhat in composition according to the fluid employed and the conditions of the experiment. In general, however, they contain a trace of protein matter and samples of various crystalloids occurring naturally in the blood plasma or artificially introduced into the circulation. Under certain conditions fluids in the peritoneal cavity can come into an apparently complete osmotic equilibrium with the blood plasma.

3. The speed of diffusion of different molecules through the peritoneum appears to vary inversely with their respective sizes. Colloids in general are not transmitted from the blood-stream into fluids in the peritoneal cavity. Colloids in solution are, however, absorbed from the peritoneal sac, although not as rapidly as crystalloids.

4. Changes in volume of hypo- and hypertonic solutions in the peritoneal cavity occur in the direction which might be predicted from a consideration of the osmotic forces at work, but never to exactly the extent demanded by the theoretical laws of pure osmosis. Absorption into the blood-stream is always greater than the expected diffusion from the peritoneal cavity, and sometimes very much greater.

5. The conclusion is drawn that while the major part of the transfer of crystalloids to and from the peritoneal cavity may be explained as being due to a simple colloidal membrane, the presence of another pathway, capable of transmitting colloids and particles as well as true solutions, and oriented only away from the peritoneal sac, must be predicated. Whether this second mode of transmission is due to some form of "vital activity" (phagocytosis, potocytosis), or whether it is due to simple mechanical filtration through intercellular spaces, actual or potential, of appropriate size, is still obscure.

It is a pleasure to remember my many colleagues and friends in Baltimore, whose interest and suggestions greatly lightened the labor of this problem. I am especially indebted to Dr. Margaret G. Smith, my collaborator in a similar research, for her help in this one; and to Professor MacCallum for permission to carry out these experiments, and for his sympathy and helpful criticisms.

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# SOME EFFECTS OF EPINEPHRIN ON THE HEART OF THE COMMON BULL-FROG (RANA CATESBIANA)

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In the course of some routine class experiments in which this animal was used to demonstrate the effect of epinephrin on the heart, some unexpected results were obtained. This investigation was begun with a view to determining to what extent these results were characteristic.

Oliver and Schäefer (1), working with Rana Temporaria found that perfusion of the heart with Ringer solution sometimes produced imperfect ventricular rhythm, the beats occurring very slowly or else falling into groups or sometimes ceasing entirely; these effects they believed were due to temporary malnutrition or lack of sufficient excitation. When suprarenal extract was added to the perfusion solution, in case of cessation of beats, spontaneous rhythm was restored; in case of slowing, the rate was increased; and in case of grouping, the number of beats in each group was increased until the groups were finally abolished in favor of uniform rhythm. This phenomenon of grouping did not occur in many of our preparations.

In case of a feebly beating heart, perfusion with suprarenal extract *seemed* to increase the strength of the beats but these workers were uncertain as to this point as most preparations were at maximum force throughout. They were, however, certain that the rate was always increased as evidenced by the shortening of the diastolic interval. Increasing the strength of the perfusion solution intensified this effect until cessation in systole finally occurred. Ringer solution alone restored the rhythm. As compared with the mammalian heart it was concluded that the frog heart is not as sensitive to suprarenal extract, a conclusion with which we can agree.

Recently Cannon (2) has proposed the use of the denervated dog heart as an indicator of adrenalin action, the criterion being the

increase of rate. Previously Brooks, McPeek and Seymour (3) had shown that when an artificial control of vasomotor action was introduced into the circulation of a dog, injection of adrenin caused slowing and augmentation of force. Artificially raising the blood pressure caused slowing and augmentation. To a certain extent this procedure had the effect of "educating" the animal to the high level. The retardation with intact innervation is common experience with mammalian hearts.

Cannon and Lyman (4) have noted that the depressor effect of adrenalin is changed to a pressor effect after pithing and conclude that this is due to opposite action of the adrenalin itself according to the state of the muscle-relaxation when tonically shortened, contraction when relaxed.

This does not agree with the findings of Snyder and Andrus (5) when studying the effect of varying H-ion concentrations of adrenalin solutions by perfusion of the terrapin medulla, leaving the heart intact. They found that if tonus of cardiac muscle was positive adrenalin would increase it; if negative, adrenalin would decrease it still further.

Working with a decapitated duck, Paton and Watson (6) found that the ventricle with intact innervation gave no augmentor response, but rather the amplitude was decreased. Collip (9) obtained similar results.

Ranson (7) reported that in the case of pithed frogs, whose hearts had been perfused for long periods with Ringer solution minus calcium until a very low efficiency was apparent, the cardiac force was restored by 1:50,000 adrenalin perfusion.

Amsler (8) found that when sympathetic endings had been paralyzed in the frog heart, adrenalin action was inverted, producing diastolic standstill and negative ino- and chrono-tropy.

Lange (10) has made the claim that adrenalin alters the irritability of muscle as an effect of altered permeability.

Gruber (11) found that adrenalin reduced high tonus produced by a variety of means.

Gruber and Kretschmer (12) were able to show that adrenalin abolished fatigue and their results were, in general, confirmed by Guglielmetti (13).

Meek and Eyster (15) reported that injections of adrenalin in all cases reflexly slowed the dog heart by vagus action. They insist that acceleration occurs as a result of direct stimulation.

Heinekamp (16) after perfusion of the terrapin medulla asserted that adrenalin stopped the heart by direct action on the cardio-inhibitory center. He also showed that a repeated dose exercised no effect. When injected directly adrenalin stimulated the heart muscle in both rate and amplitude.

*Experimental procedure:* After pithing the brain of a frog, the heart was completely denervated. With a minimum of trauma, two cannulas were inserted for perfusion as near the heart as possible in order to eliminate vasomotor effects. All other vessels were tied off. Results were recorded graphically by the suspension method. In some instances both auricles and ventricles were attached but the effects on the auricle as thus recorded were not of sufficient certainty to permit of interpretation, except the auricle was less sensitive than the ventricle and auriculo-ventricular dissociation frequently occurred. This agrees with the report of Hardoy and Houssay (14). Therefore, only the effects on the ventricle are recorded in this paper.

Two reservoirs, one for the control solution—Ringer's,—the other for the epinephrin solution, were so arranged that the perfusion pressure could be varied by shifting the height. It was found necessary to adjust the pressure to the optimum for each specimen, but, once adjusted at the beginning of the experiment, it was not altered at any time during progress. Increase of pressure beyond a certain point invariably slowed the heart, lowered tonus, and decreased the amplitude.

Siphon tubes were arranged in connection with each reservoir so that the height of the liquid inside would not affect the pressure in the heart. These tubes were connected by a Y-tube with the venous cannula so that the flow could be shifted from one reservoir to the other without mechanical disturbance of the heart as it was found to be somewhat sensitive to mechanical stimuli.

Fresh solutions of epinephrin were prepared volumetrically at the beginning of each experiment.

It was found that there were three variable factors, heart rate, amplitude of beat or force, and cardiac tonus, any one or any combination of which might be affected positively or negatively in the same preparation. Clough (17) claims only two factors, rate and amplitude or pulse pressure, each varying negatively and positively, in the case of human patients. On this account, for convenience of comparison, an index of cardiac efficiency was derived by multiplying the minute rate by the amplitude as measured in millimeters on the

graph. It is acknowledged that there are some objections to this procedure but all the graphs were taken with the same heart lever in the same position so that for comparative purposes it did not seem necessary to reduce the measurement to the actual amplitude. For purposes of simplicity cardiac tonus is recorded as percentage increase or decrease from the initial level which is expressed as 100 per cent. This was determined by taking the mean distance of the graph above the time line. This is for comparative purposes alone. By this means a greater diastolic relaxation would mean a lowering of tonus though the amplitude is greater and so, the heart more efficient; thus tonus is likely to vary inversely with the efficiency index.

*Experimental results:* A number of control experiments were run, in which perfusion with Ringer solution was continued during long periods. Table 1 shows the results of one of these which are typical of all controls. From this table it will be seen that the variations of

TABLE 1

*Experiment 8*

| TIME | HEART-RATE | AMPLITUDE | EFFICIENCY INDEX | PERCENTAGE TONUS |
|---|---|---|---|---|
| Beginning | 54 | 27 | 1458 | 100 |
| After 30 minutes | 46 | 29 | 1334 | 72 |
| After 45 minutes | 46 | 33 | 1518 | 52 |
| After 60 minutes | 46 | 33 | 1518 | 60 |
| After 110 minutes | 38 | 28 | 1064 | 81 |

rate and amplitude do not maintain the usual relationship, nor does tonus vary consistently with the other factors, decreasing in direct variation during the first 30 minutes after which efficiency increases for a time. This secondary increase occurred in every control, though its significance cannot be stated at present.

There was no evidence in any of these experiments of the results obtained by Oliver and Schäefer except that in one instance Luciani's groups occurred after 90 minutes. There was no pronounced decrease in rate until after a long period when exhaustion might be responsible.

Table 2 shows a tabulation of typical effects of perfusion with a solution of epinephrin of 1:300,000 concentration for 70 minutes. Return to Ringer solution at the end of that time did not produce any change so it is assumed that the organ had developed tolerance to epinephrin after this long period so that a change produced no effect. This accords partially, at least, with Heinekamp's findings.

Even this low concentration produced a pronounced stimulation of efficiency that is sustained above the initial level for an hour. Tonus variations are inverse throughout. After the sharp initial increase in tonus the latter falls and rises as in table 1, though the terminal rise takes place much earlier and is proportionately high at the termination. There is some evidence that this earlier rise is an epinephrin effect. The initial increase in tonus, while not occurring in every epinephrin perfusion, did not occur in *any* control experiments so it also is believed to be an epinephrin effect.

Since this is the only one of eight perfusions with this concentration that produced any epinephrin effects, 1:300,000 is probably the threshold strength for this form.

TABLE 2

*Experiment 5*

| TIME | RATE | AMPLITUDE | EFFICIENCY INDEX | PERCENTAGE TONUS |
|---|---|---|---|---|
| Ringer | 44 | 25 | 1100 | 100 |
| 2 minutes after administration 1:300000 epinephrin | 46 | 24 | 1104 | 123 |
| 8 minutes after | 42 | 36 | 1812 | 83 |
| 30 minutes after | 38 | 46 | 1748 | 79 |
| 50 minutes after | 38 | 49 | 1862 | 90 |
| 70 minutes after | 38 | 19 | 722 | 121 |

Apparently there was considerable range in sensitivity to epinephrin. In some instances the same degree of effects was produced by a concentration of 1:10,000 as was produced in this case by a 1:300,000 concentration, the range thus being 1:30.

With concentrations of 1:200,000 the results were somewhat more pronounced, also more varied. With 1:300,000, a repeated perfusion of the same concentration gave no evidence of either tolerance or sensitization having been produced by a previous application. In a comparison of two perfusions with 1:200,000 with an interval of Ringer perfusion of 10 minutes' duration, we find that in the first case the rate before epinephrin perfusion was 56, amplitude 11 mm., tonus 100 per cent. One minute after the epinephrin was turned in rate was 58, amplitude 13, tonus 100 per cent, an increase of 19 per cent in efficiency. There was gradual recovery until at the end of three minutes return to Ringer produced no immediate change but

in 10 minutes there was a gradual change similar to that produced by continuous Ringer perfusion as shown in table 1 so that the rate was 48, amplitude 13, tonus 83 per cent.

The results of a second perfusion are shown in table 3. It will be seen that efficiency and tonus vary inversely throughout. The greater effect on every factor indicates that the heart must have become sensitized since, in the early stage, efficiency is increased 70 per cent as against 19 per cent in the first trial. Furthermore, the effect on tonus is greater. But since this is a second perfusion, examination of table 4 which shows the results of perfusion of a fresh preparation with the same concentration will show that tonus after a brief fall is practically unchanged during 30 minutes while efficiency falls markedly after three minutes.

TABLE 3

*Experiment 16*

(1:200,000)

| TIME | RATE | AMPLITUDE | EFFICIENCY INDEX | PERCENTAGE TONUS |
|---|---|---|---|---|
| Ringer | 48 | 13 | 624 | 83 |
| 2 minutes after administration | 56 | 19 | 1064 | 60 |
| 3 minutes after adminstration | 53 | 16 | 848 | 95 |
| 5 minutes after adminstration | 58 | 14 | 812 | 90 |
| 9 minutes after administration | 46 | 14 | 644 | 123 |

In another case where perfusion with epinephrin had already occurred a second perfusion gave the results shown in table 5. Here the amplitude and efficiency index are increased while tonus is decreased to a small extent. The significance of the initial fall in tonus shown in tables 3 and 5 will be discussed presently. In both these cases the divergence of the two factors is apparent from the start while in table 4 they do not diverge immediately suggesting a condition similar to that existing in the control experiments (table 1). Experiment 20 (table 5) is the more typical of this group since it corresponds to the results from 6 of the 9 trials at this concentration.

Continuous perfusion for 15 minutes with 1:200,000 epinephrin in one case produced toxic effects which were reduced by perfusion with Ringer. If allowed to continue after toxic arrhythmia had set in the heart usually ceased to beat in 10 or 15 minutes. In other cases such toxic effects were produced only after a large dose of 1:1,000 solution, another piece of evidence of a wide range of susceptibility.

With concentrations of 1:100,000 epinephrin 22 experiments were run, in 15 of which there was an increase, at least temporarily, in the efficiency index, in 6 cases a decrease and in one no change though tonus increased 18 per cent. In 5 of the 15 cases only did tonus decrease as the efficiency index increased; of the six cases of decreased efficiency 4 showed an increase in tonus; so that of the total, 9 cases showed inverse variation, 2 showed no change in tonus, and 11 showed a direct variation early in the experiment.

TABLE 4

*Experiment 24*

(1:200,000)

| TIME | RATE | AMPLITUDE | EFFICIENCY INDEX | PERCENTAGE TONUS |
|---|---|---|---|---|
| Ringer | 54 | 32 | 1728 | 100 |
| 1 minute after administration | 56 | 28 | 1456 | 85 |
| 3 minutes after administration | 58 | 31 | 1798 | 98 |
| 30 minutes after administration | 24 | 29 | 696 | 96 |

TABLE 5

*Experiment 20*

(1:200,000)

| TIME | RATE | AMPLITUDE | EFFICIENCY INDEX | PERCENTAGE TONUS |
|---|---|---|---|---|
| Ringer | 46 | 29 | 1334 | 100 |
| 1 minute | 54 | 28 | 1512 | 100 |
| 25 minutes | 48 | 33 | 1584 | 91 |
| 55 minutes | 46 | 35 | 1610 | 90 |

All pronounced changes in either tonus or efficiency and tonus occurred in the first 15 minutes. Thereafter, the usual results were either the appearance of toxic effects or an apparent recovery as evidenced by the fact that a change to Ringer did not produce any particular effect after a prolonged perfusion with epinephrin.

That not all the epinephrin in solution was decomposed was shown by the fact that in nearly every case of concentrations of 1:100,000 or stronger, the perfusate when saved and perfused again gave effects comparable to those obtained from the first running but much less pronounced. This was not found in case of 1:200,000 and 1:300,000 concentrations.

In tables 6, 7 and 8 are presented some typical results from perfusion with 1:100,000 concentration. In figure 1 are shown curves from experiment 31 (table 6). There is a progressive falling off in efficiency almost from the beginning to the extent of 79 per cent and an accompanying decrease in tonus finally amounting to only 17 per cent. The progress of this experiment is further illustrated in figure 2. Comparison of these results with those shown in table 1 will show some points of similarity. Tonus falls progressively during

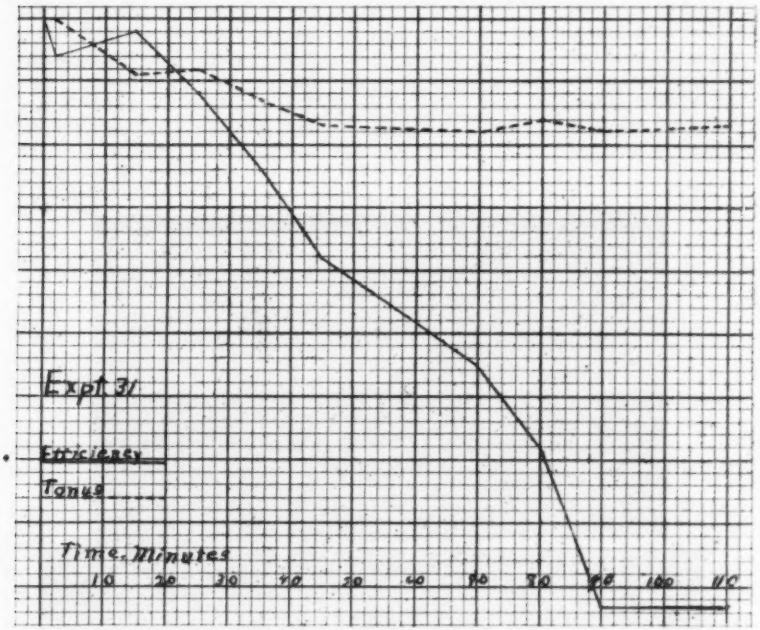


Fig. 1. Perfusion with 1:100,000 epinephrin.

45 minutes as against 45 minutes in table 1. From there on it is maintained without much variation as against a rise in table 1; there is a fall and rise in efficiency occupying about 15 minutes as against 45 minutes; furthermore this rise is not sustained as in table 2 but instead there is a sharp and progressive fall until after 110 minutes arrhythmia set in. In all, the results are suggestive of a preparation relatively resistant to this dosage of adrenalin. At any rate, there is certainly no prolonged effect from epinephrin in circulation.

TABLE 6

*Experiment 31*

(1:100,000)

| TIME | RATE | AMPLITUDE | EFFICIENCY INDEX | PERCENTAGE TONUS |
|---|---|---|---|---|
| Ringer | 30 | 38 ? | 1140 | 100 |
| 1:100000 during 2 minutes | 30 | 36 | 1080 | 100 |
| After 15 minutes | 34 | 33 | 1122 | 91 |
| After 25 minutes | 34 | 30 | 1020 | 92 |
| After 35 minutes | 30 | 30 | 900 | 87 |
| After 45 minutes | 28 | 27 | 756 | 83 |
| After 70 minutes | 28 | 21 | 588 | 82 |
| After 80 minutes | 30 | 16 | 480 | 84 |
| After 90 minutes | 26 | 9 | 234 | 82 |
| After 110 minutes | 26 | 9 | 234 | 83 |

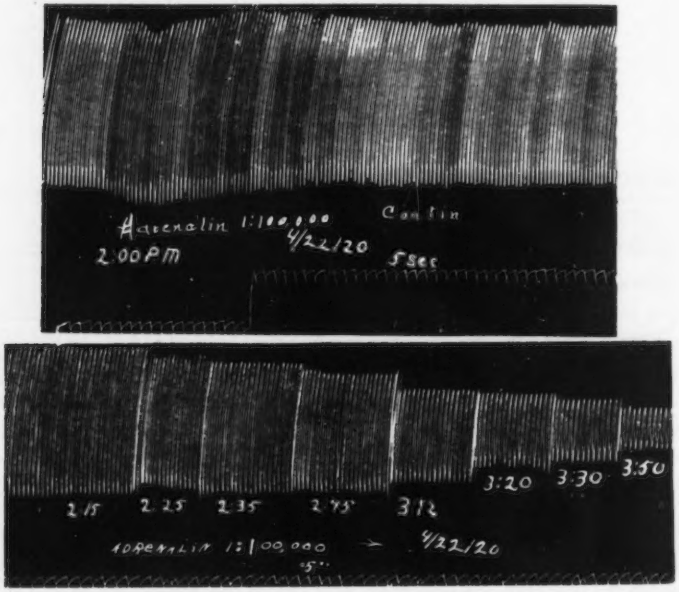


Fig. 2. Successive stages of perfusion with 1:100,000 epinephrin.

On the other hand tables 7 and 8 are much more suggestive of epinephrin effects in that in the first there is a sharp increase in efficiency followed by a sharp fall but still a maintenance of 166 per cent above initial. After 15 minutes the two factors diverge but tonus begins to rise again until it also reaches a high degree. But after a few minutes, arrhythmia set in and it was not possible to restore efficiency so that the preparation would stand a perfusion with even 1:200,000 concentration without arrhythmia in 30 seconds. This suggests sensitization. On the other hand there is also a possibility that any great degree of stimulation at any stage renders the preparation less efficient at later stages as a result of mobilization of energy in the earlier stage. And in proportion to the degree of stimulation the period of activity is shortened.

TABLE 7

*Experiment 21*

(1:100,000)

| TIME | RATE | AMPLITUDE | EFFICIENCY INDEX | PERCENTAGE TONUS |
|---|---|---|---|---|
| Ringer | 40 | 15 | 600 | 100 |
| After 1 minute perfusion | 44 | 30 | 1320 | 152 |
| After 5 minutes perfusion | 48 | 48 | 2304 | 222 |
| After 15 minutes perfusion | 44 | 41 | 1804 | 200 |
| After 20 minutes perfusion | 40 | 40 | 1600 | 261 |
| Heart block set in after 35 minutes | | | | |

In experiment 45, table 8, while there was the sharp initial drop in both efficiency and tonus already mentioned, efficiency was finally raised 116 per cent above initial and tonus continued above the initial level until a sudden onset of arrhythmia terminated the experiment after 12 minutes. It is significant that in all our experiments where one or both factors reached and maintained a high level throughout the experiment, or at least in the later stages, this toxic effect in nearly every case terminated the experiment, so that it is possible that any concentration stimulating over a long period eventually produces toxic effects. Recovery seems to occur only where the effects, of whatever nature, are confined to earlier stages. If this is merely a fatigue effect, it can be said that where epinephrin produces any stimulating effect fatigue comes on earlier.

With concentrations of 1:50,000, results were obtained of which

table 9 is typical. Tonus and efficiency vary inversely throughout, but as in the previous case, the efficiency level is maintained far above the initial; this experiment also terminated in toxic arrhythmia. The loss in tonus in this and many other cases was due to a greater diastolic relaxation, as the systolic level frequently did not vary at all. These results are typical of seven trials with this concentration.

With a still higher concentration, 1:25,000, 12 trials were made. Of these, 8 resulted in increased efficiency within the first 10 minutes,

TABLE 8

*Experiment 45*

(1:100,000)

| TIME | RATE | AMPLITUDE | EFFICIENCY INDEX | PERCENTAGE TONUS |
|---|---|---|---|---|
| Ringer | 52 | 12 | 624 | 100 |
| After 1 minute | 54 | 12 | 648 | 152 |
| After 5 minutes | 48 | 7 | 336 | 87 |
| After 6 minutes | 48 | 17 | 816 | 113 |
| After 10 minutes | 52 | 26 | 1352 | 123 |
| After 12 minutes | 54 | 25 | 1350 | 103 |

TABLE 9

*Experiment 50*

(1:50,000)

| TIME | RATE | AMPLITUDE | EFFICIENCY INDEX | PERCENTAGE TONUS |
|---|---|---|---|---|
| Ringer | 50 | 8 | 400 | 100 |
| After 1 minute | 46 | 20 | 920 | 92 |
| After 30 minutes | 44 | 23 | 1012 | 68 |
| After 50 minutes | 44 | 23 | 1012 | 68 |

usually within 3 minutes. All showed a sharp fall after the initial rise. Conversely, in the other 4 cases where there was an initial fall, there was a short rise following, which was not sustained. Of the first 8 trials, 5 showed an accompanying increase in tonus and 3 showed a decrease, while in the 4 cases showing an initial lowering of efficiency there was also an accompanying lowering of tonus. The results of four typical experiments are shown in table 10.

From these tables it is apparent that there is again a variety of results as with the lower concentrations though the majority of cases

show results favorable to a conclusion that the effect of epinephrin at this concentration is not long maintained but that the initial effect is one of increased efficiency since it is not certain that the tonus changes as detected in this way are comparable to tonus changes under normal conditions of heart activity.

As in most other experiments the results are decidedly variable, hence difficult of interpretation. There are as wide variations apparent among these four experiments at the same concentration as may be found among those at different concentrations.

TABLE 10

(1:25,000)

| TIME | | RATE | AMPLITUDE | EFFICIENCY INDEX | PERCENTAGE TONUS |
|---|---|---|---|---|---|
| Experiment 18 | Ringer | 45 | 20 | 900 | 100 |
| | 1 minute | 40 | 26 | 1040 | 87 |
| | 15 minutes | 30 | 9 | 270 | 100 |
| Experiment 59 | Ringer | 58 | 9 | 522 | 100 |
| | 1 minute | 48 | 10 | 480 | 89 |
| | 10 minutes | 50 | 11 | 550 | 95 |
| | 25 minutes | 46 | 10 | 460 | 69 |
| Experiment 63 | Ringer | 44 | 26 | 1144 | 100 |
| | 1 minute | 38 | 29 | 1102 | 129 |
| | 10 minutes | 40 | 31 | 1240 | 110 |
| | After 5 minutes in Ringer | 44 | 34 | 1496 | 100 |
| Experiment 64 | Ringer | 58 | 31 | 1798 | 100 |
| | 1 minute | 64 | 36 | 2304 | 104 |
| | 15 minutes | 54 | 39 | 2106 | 79 |

Upon perfusion with a dilution of 1:10,000 epinephrin it was found that, of 16 trials, 10 showed a pronounced increase in efficiency during the early stages which was not maintained throughout a longer period than 15 minutes except in one case where the increase was sustained for 20 minutes. There was less definite correlation between efficiency and tonus than in any other group of cases. Table 11 shows the results of three typical experiments obtained with this concentration. In experiment 54 (table 11) there is a progressive lowering of tonus after the first minute. Efficiency is increased markedly at first, progressively decreasing. Ringer solution improved both tonus and rate but did not alter amplitude.

Results of experiment 12 show a lowering of efficiency during 10 minutes with improvement during 15 minutes, but not sufficient to recover the loss. Tonus is lowered progressively to 36 per cent. It is difficult to account for the difference between these two experiments as a careful examination of all detectable factors showed no difference in the conditions. There is only the difference in susceptibility to account for the variation. It is probable that this represents a toxic effect with partial recovery after 10 minutes.

The appearance of the graph obtained in experiment 2 (table 11) is very typical of an epinephrin curve of blood pressure. Since

TABLE 11

(1:10,000)

| | TIME | RATE | AMPLITUDE | EFFICIENCY INDEX | PERCENTAGE TONUS |
|---|---|---|---|---|---|
| Experiment 54 | Ringer | 44 | 7 | 308 | 100 |
| | 1 minute | 48 | 17 | 816 | 119 |
| | 20 minutes | 28 | 15 | 420 | 36 |
| | Ringer | 34 | 15 | 510 | 55 |
| Experiment 12 | Ringer | 48 | 24 | 1152 | 100 |
| | 1 minute | 42 | 25 | 1050 | 100 |
| | 5 minutes | 38 | 27 | 1026 | 90 |
| | 10 minutes | 36 | 25 | 900 | 62 |
| | 25 minutes | 36 | 29 | 1044 | 36 |
| Experiment 2 | Ringer | 40 | 14 | 560 | 100 |
| | 1 minute | 36 | 9 | 324 | 158 |
| | 2 minutes | 22 | 13 | 286 | 191 |
| | 3 minutes | 28 | 7 | 196 | 166 |

this heart was denervated atropine was given to paralyze any vagus elements still affected. The succeeding results were slightly less pronounced but otherwise identical. This procedure was tried four times with each concentration with results consistent with those obtained in this experiment. This leads to the conclusion that a part of the typical blood pressure effects from injections of epinephrin may be due to direct effect on the heart muscle itself.

As an illustration of the varieties of effects met with in different cases, attention is called to results obtained from three successive perfusions of the same heart with the same concentration. In the first of these neither rate nor tonus is varied but there is unquestion-

ably increased efficiency—from 884 to 1300. In the second case, tonus was 18 per cent above initial and consequently there was a prolonged lowered tonus to a point 6 per cent below initial; efficiency index was decreased from 1058 to 874, largely as a result of decreased amplitude.

In the third case tonus was practically initial. Upon perfusion, amplitude only increased 1 mm. and then decreased sharply while tonus increased progressively. The efficiency index increased sharply during the first minute but decreased thereafter while tonus increased progressively. In the first case there was increased efficiency due to greater amplitude; in the third on account of increased rate.

That some preparations were less sensitive after one perfusion is apparent from a comparison of two typical cases. In one preparation already in low tonus—64 per cent—which is increased 4 per cent by a perfusion of 1:100,000, efficiency is increased from 2460 to 2666 entirely by an increase in amplitude since the rate was decreased 2 points.

A 1:50,000 dilution was used on the same heart. Here again tonus is only 58 per cent of initial and is unchanged except for a slight fall during the first few seconds. The rate fell from 64 to 60 and the amplitude increased from 32 to 33 giving an index lowered from 2048 to 1980, a 4 per cent decrease as compared to an 8 per cent increase with the more dilute solution. A change of this sort is difficult of interpretation unless it be supposed that the heart developed a tolerance as a result of previous perfusions, to a sufficient extent to get the effect from this second solution of an extremely dilute one.

It is apparent even upon casual consideration that much of the peculiarity of response is dependent upon the species of the subject. Most workers have found fairly uniform results in all other forms. These experiments further confirm the findings that the frog is comparatively less responsive to epinephrin than mammals.

Tonus waves in the graphs taken from this heart were never very pronounced and invariably disappeared toward the later stages of the experiments no matter to what treatment the heart was subjected. This would indicate the possibility of a smaller amount of smooth muscle fibers in the cardiac musculature of the frog than in the chelonian heart.

It has been freely assumed that epinephrin acts on the myoneural

junction of the nerve fibers. But since, in this series of experiments, atropine was followed by only slightly less pronounced effects than had already been obtained, it must be that the drug acts in fact directly on the musculature itself. There was no indication of an inversion such as Amsler reported. If it is true as Lange claims that epinephrin alters the irritability of cardiac muscle by a change of permeability, it is more difficult still to understand the contrary results obtained.

That the changes were not mechanical was shown by shifting the flow of Ringer solution from one reservoir to the other, by slightly raising or lowering the temperature and by slightly compressing the influent tube. None of these manipulations had any effect.

In consideration of the results of continuous perfusions with Ringer solution, it is suggested that the initial fall in the efficiency curve may be due to lack of adaptation to the changed condition, an adaptation or "education" later consummated. The final fall is, of course, due to experimental limitations.

As to whether the relationship between the tonus and efficiency curves is direct or inverse, this depends on whether tonus changes are dependent upon increased relaxation, which would increase amplitude, or on increased systole which would again increase amplitude, or whether there is an increase in amplitude, as in some instances, without any change in tonus. The factors that determine the direction of the change are not apparent. More work must be done to determine this point.

A survey of all the results shows that the force of the heart beat was more frequently affected and to a greater extent than the rate. This being true, it is apparent that where rate and amplitude vary inversely, there is merely mechanical adjustment. But where the two vary together, there is actual stimulation or retardation as the case may be.

It is also noticeable in the control experiments that amplitude did not vary greatly. Since this is true and since in all experiments with epinephrin this is the factor of the two that is most affected, it is assumed that the primary effect of epinephrin on the heart of this form has to do with changes of force.

Since the most pronounced changes occur in the early stages of most experiments, our results in a remote way support the theory of emergency function of epinephrin. Wherever the heart responds in a way that can be interpreted as physiological and not pathological,

continuous application of epinephrin has no very noticeable effect. Lower concentrations which produced any evidence of stimulation were usually unaffected by a return to Ringer so it is assumed that epinephrin at that stage was not effective.

It is possible, of course, that some of the variable effects produced, are due to the "opposite action" of epinephrin as suggested by Cannon and Lyman. In fact we are inclined to hold to this explanation as all facts point to it as logical.

## SUMMARY

1. Great variability in the response of the denervated heart of Rana Catesbiana to perfusion with epinephrin is a peculiarity of the species.

2. The auricle is less sensitive than the ventricle. Auriculo-ventricular dissociation frequently occurs.

3. The bull-frog heart is comparatively less responsive than the mammalian.

4. The threshold concentration is probably about 1:300,000 since this concentration was only occasionally effective.

5. Since solutions of 1:10,000 concentration gave results comparable to those with 1:300,000, there is evidently a wide range of sensitivity.

6. With concentrations above 1:200,000 a reperfusion of the same solution gave some effects typical of a weaker solution showing that complete decomposition had not taken place.

7. There were three variable factors, rate, force and tonus; but in most instances, epinephrin increased the efficiency of the heart more frequently by augmenting the force than by increasing the rate.

8. With stronger concentrations repeated perfusion showed evidence of increased tolerance in most cases, occasionally increased sensitivity. This effect was not apparent with more dilute solutions.

9. If the perfusate was of sufficient strength to cause sustained stimulation of a high degree the experiments terminated in a toxic arrhythmia. Ringer solution seldom restored the normal rhythm.

10. Where toxicity did not occur the most pronounced effect, whether of stimulation or inhibition, occurred relatively early.

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# THE HEAT LIBERATED BY THE BEATING HEART

## Fourth Communication

## *A Note on the Energy Exchanges*

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*I.* In recent work Meyerhof (2) has redetermined the heat of combustion of lactic acid, and has concluded it to be 3615 calories per gram in 74 per cent concentration and 3601 calories in dilution such as obtains in frog's muscle. As pointed out by Hartree and Hill (4), this number is nearly the same as that gotten by Emery and Benedict (1). It is doubtless nearer the true value than that of Louguinine's, the one I have lately used in my calculations of the probable sources of heat produced in the terrapin's heart (6). It would be accordingly of interest to know what difference this new number would make in my estimates. In what follows the heat exchanges are given in aliquot parts of the reactions involving 1 gram of lactic acid instead of a molecular weight as was done in the former communication (6, p. 281).

For the anoxidative (systolic) stage of the process we have then 0.9 gram glycogen going over to 1 gram lactic acid. The corresponding heats involved are 0.9 × 4191 calories for the glycogen (Stohmann, Schmidt, 1894)[1] and 3601 for the lactic acid. The difference between these two heats, 171 calories, it is assumed, is freed by the breakdown of glycogen to lactic acid, and represents the heat of chemical reaction for the so-called anoxidative phase of a muscle contraction (3) which I take to last during the systole of the heart beat.

During the oxidative phase of the muscle action or the period of recovery, which I take to include the periods of diastole and pause of

[1] Emery and Benedict made only two determinations of the heat of combustion of glycogen and these vary somewhat. There seems to be no good reason to adopt their number rather than Stohmann's nor even to adopt a mean of the figures of the two authors.

the cardiac cycle, it is further assumed that, as in skeletal muscle, $\frac{1}{5}$ of the lactic acid is oxidized and $\frac{4}{5}$ are built back to glycogen (3).[2]

Supposing 1 gram represents the total lactic acid produced, then $\frac{1}{5}$ of 3601 or 720.5 calories are liberated by oxidation. On the other hand $4 \times 720.5$ or 2882 calories will represent the heat equivalent of the $\frac{4}{5}$ gram that are built back to glycogen. The amount of glycogen rebuilt being 0.72 gram, its heat equivalent is $0.72 \times 4191$ or 3017.5 calories. This latter endothermic process will absorb $3017.5 - 2882$, or 135.5 calories of heat. The heat given up by the whole of the oxidative phase thus becomes $720.5 - 135.5$ or 585 calories. The sum of all the chemical heat for both anoxidative and oxidative phases, or for the whole cardiac cycle, is finally $171 + 585$, or 756 calories. For these figures the ratio of the heat of the (anoxidative) systolic period to that of the whole heart cycle may be put as 171/756, or as

$$1{:}4.421, \qquad (1)$$

In my measurement of the heat produced during the various phases of the heart-beat the amount per gram muscle was found to be about 0.0003 calorie for the systolic (anoxidative) phase and $0.00066 + 0.00034$ calorie respectively for the two outbursts of heat during the periods of diastole and pause, the oxidative phase. (For these determinations see my paper, (6), p. 282.) The total output of heat for the whole oi

[2] On page 141 of his *IV^ten Mitteilung* under the caption, "Über die chemischen Vorgänge im intakten Muskel," Meyerhof (3) finds under favorable conditions that recovery from fatigue goes on in the ratio,

$$\frac{\text{Mols. of lactic acid disappeared}}{\text{Mols of lactic acid burned}} = \text{nearly } 4$$

That is, out of 5 parts of the acid formed one is burned and 4 are rebuilt to glycogen. On page 141 of this same article he writes the probable equation expressing this reaction, and in a footnote says, "Ich halte die jetzige Formulierung fur die präzisere: auf 5 Glucose molecule verbrennt eines." (In the equation glucose is made the immediate source of lactic acid). In his more recent work (Pflüger's, 1922, excv, 71) Meyerhof expresses the conviction that in no case could the ratio in question be greater than 4:1 in intact muscles. Hartree and Hill, however (4, p. 378) on the basis of new experiments with muscle in pure $O_2$ conclude that "only from one-fifth to one-sixth of the lactic acid is oxidized—the remainder is 'restored' ". I quote all this to indicate to the reader the source of the evidence for choosing this 1:4 ratio rather than the 1:3 ratio appearing in Meyerhof's earlier work,—Pflüger's Archiv., 1920, clxxxii-clxxxv.

the cardiac cycle thus becomes 0.0013 calorie.[3] The ratio of that produced during the systole to the total amount therefore is $\frac{3}{13}$, or

$$1{:}4.333, \qquad (2)$$

This latter ratio of the observed heats is a little less than 2 per cent of that demanded by the heat equivalents of the probable chemical reaction involved, as shown in ratio (*1*).

A similar comparison of the energy values for the oxidative phase (period of diastole and pause) and the sum for the whole heart beat indicates that for the chemical reactions the ratio is 585/756, or

$$1{:}1.292, \qquad (3)$$

and for the observed heats produced (0.00066 + 0.00034) / 0.0013, or

$$1{:}1.30, \qquad (4)$$

Here the latter ratio varies 0.61 of 1 per cent of that demanded by the chemical reaction, or ratio (*3*).

Returning now to ratios (*1*) and (*2*) which compare the heat measured during systole with that demanded by the thermochemical exchange, it will be noted the numbers indicate that the heat observed is a little more than what is demanded by the chemical action alone. In my former paper where I made use of Louquinine's heat equivalent of lactic acid, and where I used my own data differently from what I do here (see footnote 3 below), these two ratios were widely separated. Nevertheless I undertook to account for the excessive heat during the anoxidative phase (6, p. 83) by assuming that some of it arose from a thermo-elastic effect. I based my calculation of this effect upon data of Hill and Hartree (5). In the present case the ratios appear to deviate

[3] The consideration that led me (6) to adopt 0.0011 calorie as the total heat rather than the sum 0.0013 calorie, of the actual observed positive heat waves, was in large part the small negative heat waves that often were observed to follow the two post diastolic positive waves. This in a sense was an attempt to take the algebraic sum of the + and − waves, an attempt that was not well founded inasmuch as, if the great diastolic negative wave representing a possible absorption of 0.0011 calorie had been included in the operation, the result would have been practically zero output of heat by the heart per beat! Since publishing that paper I have concluded that a truer interpretation of the waves therein observed will be gotten by ignoring (for the present at least) all the negative waves and considering only the three positive waves. The points brought out in the present paper seem fully to justify this later interpretation of the thermocardiographic waves.

no more than may be accounted for by the probable experimental error. Nevertheless it is of interest to recalculate the thermo-elastic effect as it may have appeared in my experiments.

Let $x$ represent the heat of thermo-elastic origin and let us assume that this heat substracted from the observed systolic heat when compared with the total heat observed (0.0013 calorie) would give a ratio exactly equal to that demanded by the heats of thermochemical origin. Then

$$\frac{0.0003 - x}{0.0013 - x} = \frac{1}{4.42}$$

whence the heat of elastic origin appears as

$$x = 0.6 \times 10^{-6} \text{ calorie}, \tag{5}$$

Now from observations of Hill and Hartree (5) I calculated (6, p. 283) that 1 gram load of tension exerted by 1 gram of muscle produces a thermo-elastic effect of 0.8 to $2.4 \times 10^{-6}$ calorie.[4] The average maximum tension of the heart muscle in my experiments was 29.6 grams; the average weight, 2.3 grams. This is 29.6/2.3 or 12.9 grams tension exerted per gram muscle (see table 2, p. 265, my article, *loc. cit.*). Taking the lower thermo-elastic value given above (since heart muscle is a weaker muscle than skeletal) the heat produced by this elastic strain, $x_1$, would be $12.9 \times 0.8 \times 10^{-6}$, or

$$x_1 = 10.3 \times 10^{-6} \text{ calorie}, \tag{6}$$

This is greater than the value of $x$ in equation (5), i.e., the probable amount of heat in excess of that demanded by the chemical action during systole, or the anoxidative phase of muscle action; and indicates that heat from this source in the thermocardiograms may not be negligible.

Let us consider therefore $1 \times 10^{-5}$ calorie, as the true value of $x$, that is, as the heat observed during the anoxidative phase due to elastic effects. Then the heat of thermo-chemical origin may be put at 0.0003 − 0.00001, or 0.00029 calorie and the total thermochemical

[4] These observations are summed up by Hill and Hartree (5, p. 171) by the statement, " . . . . . a load of 150 grm., added to a pair of sartorius muscles of *Rana Temporaria*, weighing, say, 150 mgr., would raise the temperature by something of the order of 1 to 3 thousandths of a degree, this being a fifth to a half of the rise produced by a strong twitch."

heat of the entire heart cycle becomes 0.0013 − 0.00001, or 0.00129 calorie. The ratio then of the observed heat of the anoxidative phase to the heat of the whole of the chemical reaction is 0.00029/0.00129, or

$$1{:}4.44, \tag{7}$$

This proportion is nearly ½ of 1 per cent of that demanded by the theory as shown above, namely,

$$1{:}4.42, \tag{1}$$

As for ratios (3) and (4) in which observed heats are compared with the theoretical heat of chemical reaction during the oxidative phase of the heart beat, it will be noted that here the difference between them indicates that either the observed heat of the oxidative phase is larger or the observed heat of the whole heat cycle is smaller than the heats demanded by the probable chemical action. This deviation also lies doubtless well within the limits of experimental error, nevertheless let us proceed to correct for the assumed thermo-elastic effect here also. The thermo-elastic effect is a part of the total heat measured (represented by the second term in this ratio); the ratio for the anoxidative phase then may be stated thus: The observed heat of the oxidative phase is to the observed chemical heat of the whole heart cycle as

$$\frac{0.00066 + 0.00034}{0.0013 - x} = \frac{0.001}{0.0013 - 0.00001}$$

or as,

$$1{:}1.29, \tag{8}$$

This proportion is thus exactly the same now as that demanded by the theory as shown above, namely,

$$1{:}1.29, \tag{3}$$

It thus appears that if one subtracts the heat of elastic origin, that probably makes up a part of the heat production during the heart's systole, then the ratios of the remaining heats,

*a*, of the systolic heat to that of the whole heart cycle, (1), and

*b*, of the diastole and pause to that of the whole heart cycle, (3), more closely approximate the corresponding ratios of the chemical changes going on in terrapin heart muscle during the beat, if these changes are

assumed to be the same as those discovered and described by Meyerhof for frog's muscle during contraction and recovery.

*II.* It is of further interest to recalculate some of the absolute equivalent values that are concerned with the post-systolic heat production.

From a number of sources it was shown that surviving mammalian hearts utilize about $2.9 \times 10^{-7}$ gram dextrose per gram muscle per beat (6, p. 280). If we assume that the dextrose was burned in the form of lactic acid then its heat equivalent (for the oxidative phase) would be $2.9/10^{-7} \times 3601$, or 0.00104 calorie, and ought to appear during the diastole and pause (recovery period) of the heart beat. As was stated above the heat production of this period in the terrapin hearts was observed to be 0.00100 calorie, a number that comes within 4 per cent of the heat equivalent of the mean sugar consumption in surviving mammalian hearts. Considering the diverse nature of the species of animal involved, the approximation of the two numbers is more striking than their disparity.

Turning to the observed $O_2$ consumption by isolated mammalian hearts I calculate from Barcroft and Dixon (7) that in "normal" blood-fed hearts $1.7 \times 10^{-7}$ gram $O_2$ per gram muscle per beat is consumed, whereas from Rohde's experiments (8, 1910) on cat hearts fed with oxygenated Locke solution the amount is about $3.5 \times 10^{-7}$ gram. An average of these two figures is $2.6 \times 10^{-7}$ gram. Assuming as was done above that the heat of the post-systolic phases observed for my terrapin hearts, 0.001 calorie, arose from the oxidation of lactic acid, then the amount of $O_2$ required would be $0.001 \times \frac{96}{3601 \times 90}$, or $2.9 \times 10^{-7}$ gram per gram muscle per beat. Here the variation between the two methods of arriving at $O_2$ consumption is 10 per cent; but the variations found by direct determination differ still more (compare Rohde, 8, 1910 and 1912).

Or again, assuming that all this $O_2$ is used up in burning lactic acid, the heat produced thereby would be

$$\frac{90 \times 2.6 \times 10^{-7}}{96} \times 3601, \text{ or } 0.00086 \text{ calorie}$$

an amount that is 14 per cent less than that observed in the recovery phases of the thermocardiograms.

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